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ANTAGONISM OF INHIBITORY ACTION OF ADRENALIN AND DEPRESSION OF CARDIAC VAGUS BY A CONSTITUENT OF CERTAIN TISSUE EXTRACTS

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INTRODUCTION

The presence of the so-called "peristaltic hormones" in various tissues has frequently been demonstrated. Enriquez and Hallian (1) found that extracts of the gastric and intestinal mucosa contained a powerful stimulant for the intestinal musculature. Fawcett, Rahe, Hackett and Rogers (2) found that protein-free aqueous extracts of the liver, pancreas and spleen, and of the pituitary, pineal, thyroid, parathyroid, thymus and adrenal glands caused a characteristic stimulatory effect upon the unstriated muscle fibers of the cat's uterus. This stimulatory effect they found to be antagonized by adrenalin. Abel, Pincoffs and Rouiller (3) have prepared from the intestinal mucosa of the pig a water-soluble powder which showed great physiological activity in stimulating an intestinal or uterine strip or in causing the pancreas to secrete freely.

Stern and Rothlin (4) have recently shown that water extracts of liver, kidney, thyroid, lung, muscle, thymus, bone-marrow, bile and blood, contain two kinds of substances, one causing contraction of smooth muscle, the other relaxation. The former substances only, they hold, are present in lymph glands, adrenal bodies and spleen. They found that the constrictor substances were soluble in alcohol and insoluble in ether, while the depressor substances were insoluble

in alcohol but soluble in ether. Both types of substances resisted boiling, but the former were destroyed by alkali while the latter were not. Matsumoto and Macht (5) found that the vas deferens of laboratory animals responds with contractions only to large doses of gland extracts. Cow (6) has found that the inhibitory action of adrenalin upon the uterus of the guinea pig, rat and non-pregnant cat is reversed by previous treatment with pituitrin.

In view of the fact that associated with shock following severe trauma there is always the possibility of certain constituents of damaged tissue entering the blood stream, thus giving a condition somewhat analogous to the intravenous injection of tissue extract, a further investigation of the effects of extracts of various tissues, prepared in a variety of ways, upon isolated organs was undertaken.

The general observations that aqueous extracts of various tissues contain certain principles which increase the tonus of smooth muscle were confirmed. Certain additional phenomena of considerable interest were manifested in several experiments; it is these latter that will be dealt with subsequently.

EXPERIMENTAL

The action upon isolated mammalian intestine and uterus, and on the heart of the terrapin, of tissue extracts prepared in the following ways was studied. Extracts made by grinding fresh tissue in the mortar with pure sand and normal Ringer-Locke's solution and subsequently centrifuging. Extracts made by boiling fresh tissue, previously macerated, with several volumes of distilled water, filtering and concentrating the filtrate on the water-bath to an aliquot volume of the original tissue used, thus rendering the extract practically isotonic. Extracts made by extracting the water-bath dried, water-soluble fraction with 98 per cent alcohol, filtering and evaporating the filtrate to dryness on the water-bath and taking up the residue in Ringer-Locke's solution. Extracts made from tissue previously dried at 110°C. to 120°C. Extracts made by boiling desiccated glandular tissue supplied by Armour & Company, Chicago, with distilled water in amount equal to the water content of the original tissue, and filtering. The desiccated thyroid body, parathyroid gland, thymus gland, pancreas, testes, corpus luteum and pituitary body (anterior and posterior lobes) were treated in this manner.

The uterus and intestine of the rat were used most extensively in this investigation, but preparations were also made from the guinea

pig, rabbit, dog, mouse and cat. Preparations were suspended in normal Ringer-Locke's solution contained in a glass perfusion chamber of 40 cc. capacity which was immersed in a water-bath kept at 38°C. Oxygen was continuously bubbled through the perfusion fluid. Movements of the isolated smooth muscle strip were recorded on the drum by means of a Harvard heart lever. The extracts were brought to body temperature previous to being added to the saline bath. The effect of tissue extracts upon the heart of the terrapin was determined in one of two ways. The brain of the animal was pithed, the heart exposed, and the apex of the ventricle connected by a silk thread to a heart lever. Both vagi were then dissected free in the neck and their response to rapid make and break shocks determined. The extract was then injected directly into the ventricle or else it was added to Ringer-Locke's fluid and the heart perfused by means of a cannula placed in the left abdominal vein, the fluid escaping through the cut aorta. Intravenous injections of certain extracts were also made into dogs to determine the effect upon the cardiac vagus and the response to adrenalin administered by the intravenous route.

RESULTS

It was found, of the tissues investigated—heart, lung, spleen, liver, brain, cord, posterior spinal root ganglia, pancreas, skeletal muscle, testes, small intestine, gastric mucosa, corpus luteum, and thyroid, parathyroid, pituitary (anterior and posterior lobe), and thymus glands—that all contained in greater or less amount substances which caused increased tonus, amplitude and rate of contractions of strips of duodenum, small intestine, and virgin and gravid uterus. The substances were not destroyed by boiling and were soluble in alcohol. Depressor substances were also present but the usual effect of the addition of 1 or 2 cc. of an extract to the Ringer-Locke's solution in the bath was an immediate increase in the tonus of the smooth muscle strip taken from the duodenum, jejunum or ileum. It was more difficult to demonstrate stimulatory action in the case of the uterus owing to the fact that so often excellent tonus and rhythmic contractions were manifest prior to the addition of the extract. In the case of the extract of pancreas the immediate effect upon the duodenum was a decrease in the general tonus which, however, was quickly recovered and definite stimulation was manifested (fig. 1, *a*). Pancreatic juice obtained from a cannula placed in the pancreatic duct of a dog did not have this effect. Again in the case of aqueous extracts of oven-

dried tissues studied (brain, cord, posterior root ganglia, duodenum, heart, pancreas, spleen, skeletal muscle) a deviation from the general rule was noticed. The primary effect of such extracts upon the intestinal musculature was typically that of adrenalin (fig. 1, *b*). The primary depression which was caused by the addition of such an extract was, however, followed at once by an increase in the general tonus. These latter extracts had little effect upon the uterus unless used in fairly large amount, in which case decreased tonus resulted (fig. 1, *c*).

It was found that extracts prepared from the heart, pancreas, corpus luteum, pituitary body (both anterior and posterior lobes), testes, and thyroid, parathyroid and thymus glands in addition to causing intense stimulation of the uterus antagonized the inhibitory action of adrenalin on such uteri as are normally inhibited by this latter substance, as in the case of the rat and guinea pig (both virgin and gravid), and the virgin rabbit and dog (figs. 1, *d;* 1, *e;* 2, *a;* 2, *b;* 3, *a*). This phenomenon is strikingly similar to that, recorded by Cow (6), of the reversal of the inhibitory effect of adrenalin on the uterus of certain animals by previous sensitization with pituitary extract. The results of Cow (6) were in part confirmed by the writer. Extracts of tissues studied other than those mentioned above, were not as effective in this regard. Partial antagonism was obtained by extracts of lung, gastric mucosa and liver, but in these instances the amounts of extracts used were relatively quite large. This antagonism of the inhibitory action of adrenalin by extracts of certain tissues was effective only as long as the extract was actually present in the saline bath. The inhibitory response to adrenalin returned in each case after the uterine strip was washed free from the extract (fig. 3, *a*). It was difficult to determine absolutely whether the stimulant action of adrenalin on the gravid uterus of an animal such as the rabbit was antagonized by extracts like that of the spleen, as the tonus produced by the addition of the latter was practically maximal; however, the writer is of the opinion that the stimulatory effect of adrenalin is not antagonized on such uteri as are normally stimulated by it.

The antagonism of the inhibitory action of adrenalin on certain uteri by extracts above noted was not demonstrated in the same decisive manner in the case of the intestinal musculature. Partial antagonism was, however, clearly demonstrated on different occasions by the raising of the threshold for adrenalin inhibition of the duodenum or jejunum of the rat by extract of spleen or heart (fig. 4, *a*).

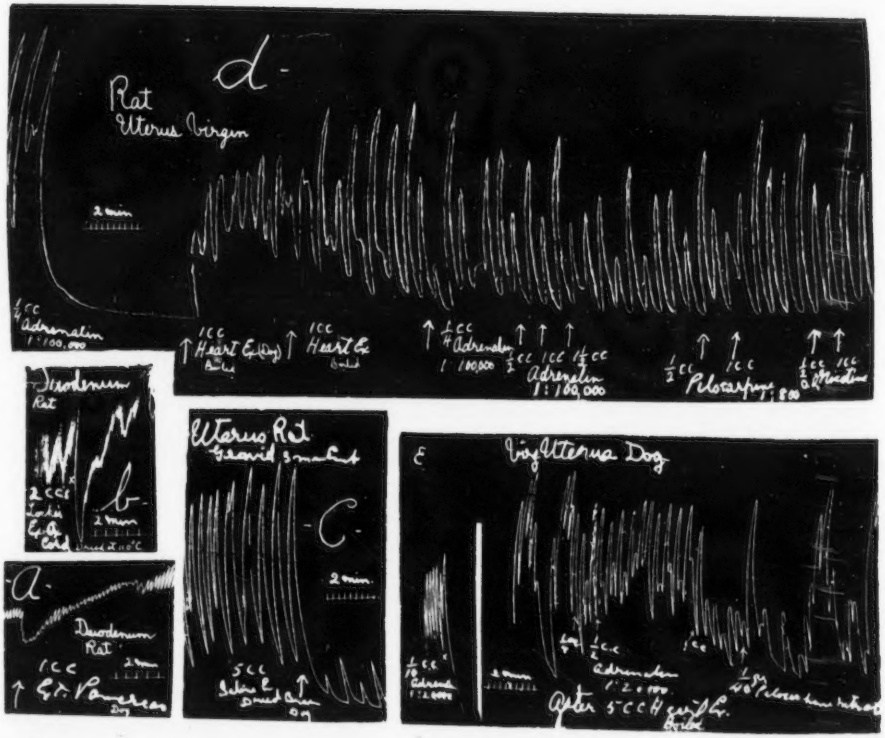

Fig. 1. *a*. Effect of 1 cc. of saline extract of fresh dog's pancreas upon isolated duodenum of the rat.

b. Effect of 2 cc. of extract of ox cord dried at 110°C. upon isolated duodenum of rat.

c. Effect of 5 cc. of extract of brain of dog dried at 110°C. upon isolated gravid uterus of rat.

d and *e*. Antagonism of inhibitory action of adrenalin by heart extract: *d*, non-gravid uterus of rat; *e*. non-gravid uterus of dog.

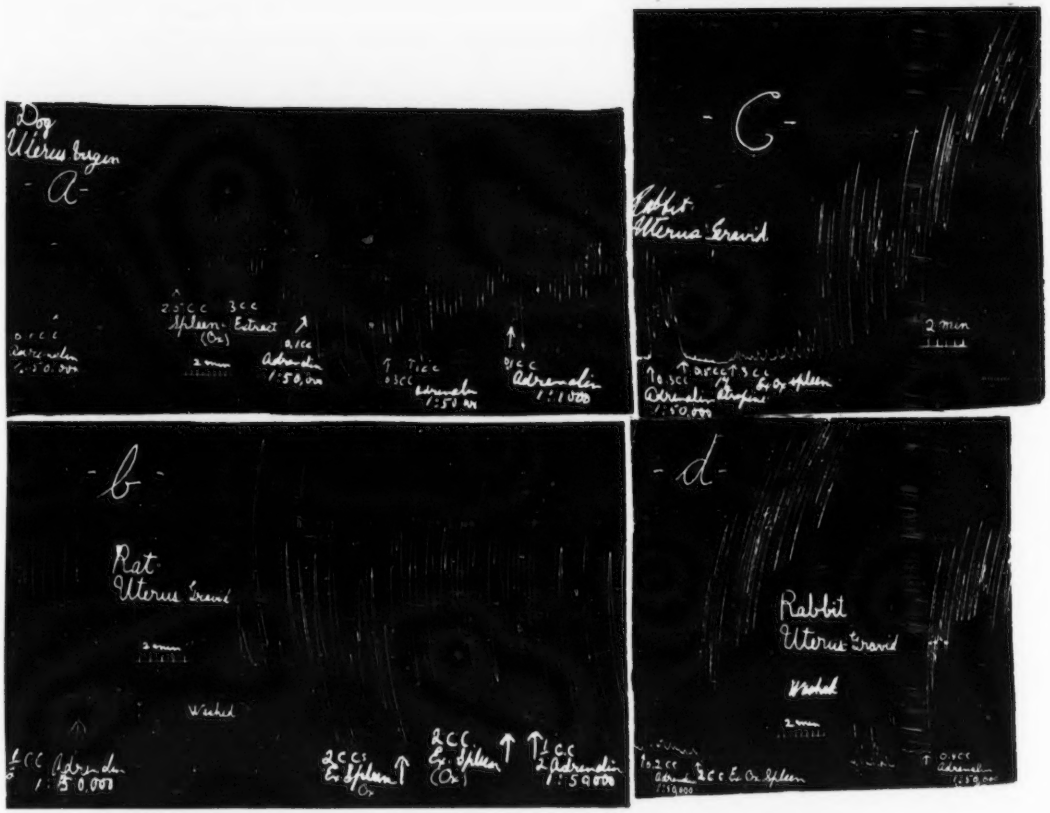

Fig. 2. *a* and *b*. Antagonism of inhibitory action of adrenalin by extract of ox spleen: *a*, non gravid uterus of dog. *b*, gravid uterus of rat.

c. Action of splenic extract upon atropinized gravid uterus of rabbit.

d. Action of adrenalin on gravid uterus of rabbit after splenic extract.

d. Action of adrenalin on gravid uterus of rabbit after splenic extract.

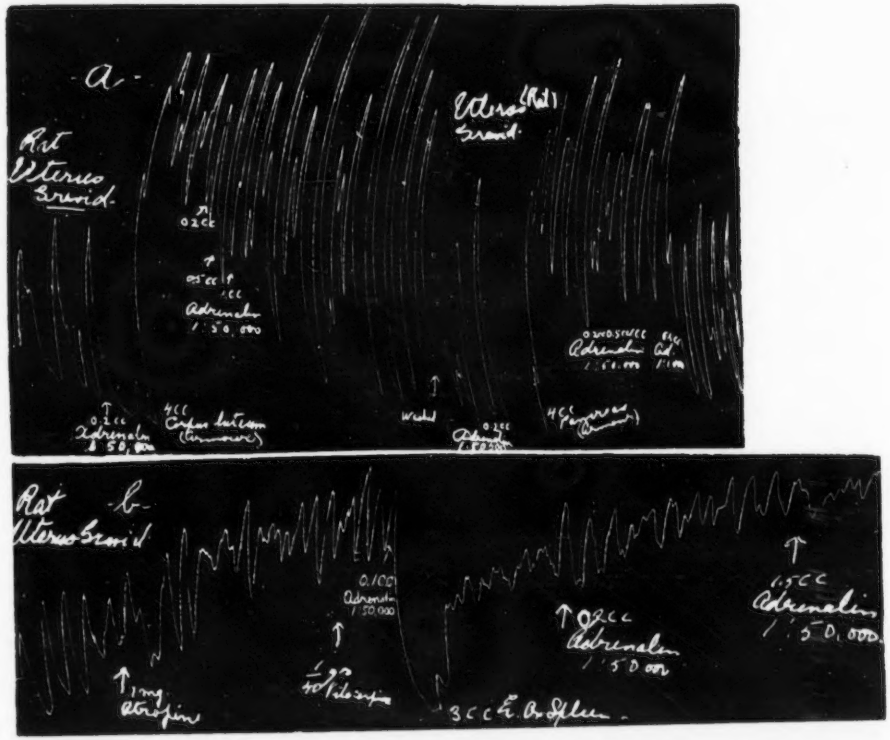

Fig. 3. *a*. Antagonism of the inhibitory action of adrenalin on the gravid uterus of rat by extract of corpus luteum and pancreas (Armour & Co., Chicago).

b. Effect of adrenalin on the gravid uterus of the rat before and after the addition of extract of ox spleen.

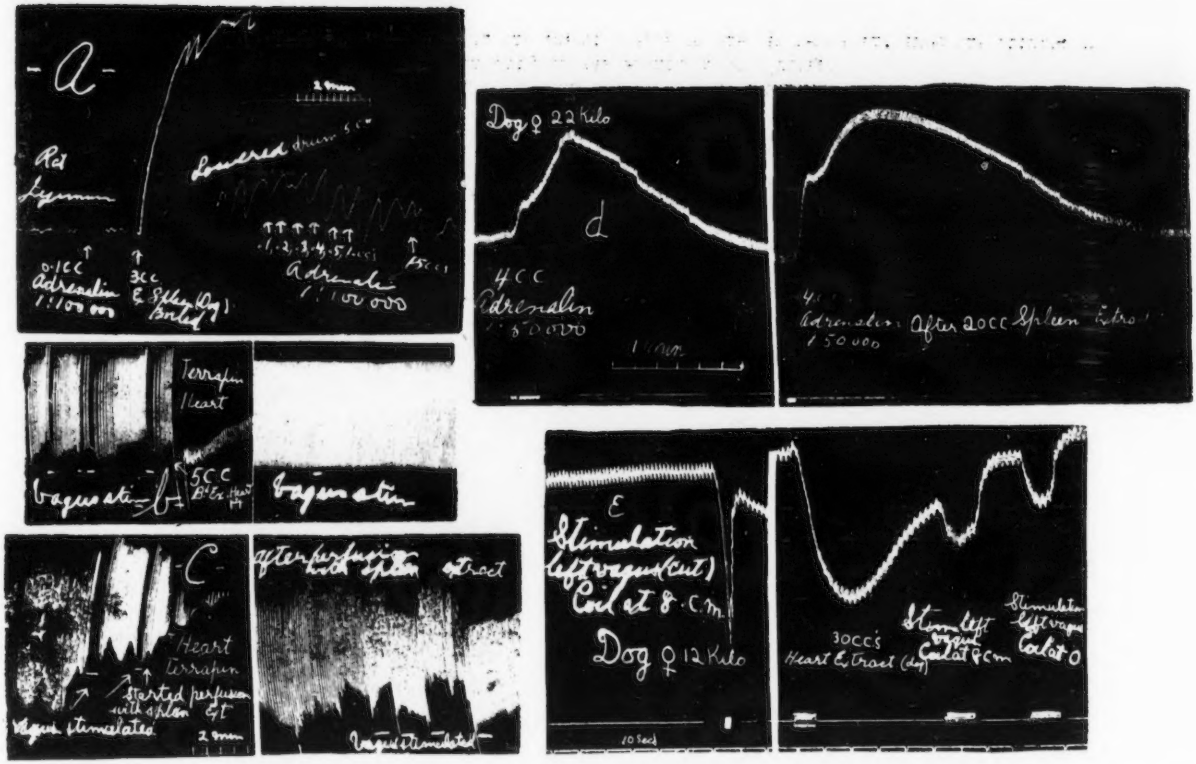

Fig. 4. *a*. Antagonism of the inhibitory action of adrenalin upon jejunal musculature of rat by 3 cc. of boiled extract of dog's spleen.

b and *c*. Complete depression of cardiac vagus of terrapin to electrical stimulation by: *b*, extract of pig's heart; *c*, extract of spleen of ox.

d. Effect of adrenalin before and after extract of spleen.

e. Depression of cardiac vagus of dog by heart extract.

It was found, when splenic extract was injected intravenously into a dog, that the rise in blood pressure produced by a definite dose of adrenalin was slightly augmented, and also the high pressure was longer maintained (fig. 4, *d*). Electrical excitation of the vagus which, prior to the injection of the extract into the ventricle or the perfusion of the same through the heart by way of the anterior abdominal vein caused complete inhibition of the heart, was found to be without effect after treatment with the extract (figs. 4, *b* and 4, *c*). The extract caused at first considerable depression of the myocardium but spontaneous beats continued and a later effect was in some instances increased amplitude of contraction with little change in the rate. The response of the heart to excitation of the vagus did not return till some time after the injection of the extract. If however the extract was washed away by perfusing the heart with a quantity of modified Ringer-Locke's solution, the heart as a rule responded to vagus stimulation after a period of from fifteen to thirty minutes' washing. When the stimulation of the vagus with the induced current from the secondary coil of a Harvard inductorium set at zero with a 6-volt current running in the primary failed to produce inhibition, injection of pilocarpine into the heart caused partial inhibition marked by a decrease in the rate of contraction. The injection of 30 cc. of heart extract, made by boiling the macerated tissue with distilled water and filtering and concentrating the filtrate till it was practically isotonic with blood serum, into the left jugular vein of a 12 kilo female dog under ether anesthesia made complete vagus inhibition temporarily impossible to obtain (fig. 4, *e*). Depression of the vagus in the intact dog by splenic extract was not so obtained.

The stimulating action of splenic extract upon uterine muscle was manifested after the tissue had been atropinized (figs. 2, *c* and 3, *b*). Adrenalin caused complete inhibition of the atropinized gravid uterus of the rat, but this was antagonized by splenic extract and further treatment with adrenalin was without effect (fig. 3, *b*). It has been found that the water-alcohol soluble fraction of the splenic extract remains active as regards its ability to antagonize the inhibitory action of adrenalin on certain uteri and to depress the cardiac vagus of the terrapin after it has been freed from certain constituents by treatment with basic lead acetate and silver nitrate in alkaline medium. Also the lead and silver precipitates, after removal of the lead and silver respectively, dissolved in Ringer-Locke's solution failed to produce any such effect.

c. Depression of cardiac vagus of dog by heart extract.

An acid-alcohol extract of the water-bath dried lipoid-free water-soluble fraction of ox spleen, treated with anhydrous ether, gave a brownish-red precipitate. This was freed from ether and dissolved in Ringer-Locke's fluid. It was found to have no stimulating action on the uterus of the rat and it did not antagonize adrenalin. A slight stimulatory effect was produced upon the isolated duodenum of the rat by this extract. Crawford (7) found that the active principle of the pituitary gland was removed from acid-alcohol extracts by anhydrous ether.

DISCUSSION

The fact that extracts of several tissues antagonize the inhibitory action of adrenalin on strips of isolated uterus which are normally inhibited by it, and also depress the cardiac vagus of the terrapin, suggests that the same constituent or constituents are present in each of these tissues. The point of action in each case is undoubtedly in the peripheral inhibitory nervous mechanism of the organ concerned, probably the myoneural junction itself. Nicotine is without effect after the action of adrenalin on the uterus has been abolished and after the vagus has been completely depressed in the heart of the terrapin. Pilocarpine may have an appreciably stimulatory effect on the uterus after the addition of certain extracts and it is still mildly active on the heart after electrical excitation of the vagus fails to inhibit. Atropine causes slight depression of the uterus after heart or splenic extract have acted and it abolishes the slight action of pilocarpine on the heart of the terrapin with vagus depressed.

As the stimulatory action of splenic extract is still manifested on the atropinized uterus of the rabbit and rat, it is probable that the stimulatory effect is due to the direct action upon the smooth muscle fiber. It is also true that some of the stimulatory effect of certain tissue extracts upon strips of smooth muscle may be due to a depression of the local inhibitory mechanism.

The depression of the inhibitory mechanism which is so clear-cut in the case of certain uterine preparations and of the heart of the terrapin is of interest in another way. The inhibitory nerves of the heart are of the parasympathetic type while those to the uterus are mixed, but in those instances where adrenalin inhibits at all times it is to be supposed that the inhibitory fibers are largely of true sympathetic derivation. Here then (granting that it is the same type of principle which is active in each case) is an instance of certain tissue constit-

uents acting selectively upon the inhibitory nervous apparatus of a tissue irrespective of whether it is of the sympathetic or parasympathetic type.

As the intravenous injection of splenic extract augments the pressor effect of a definite amount of adrenalin, it may be that some part of the vasodilator mechanism which is stimulated by adrenalin (8) is depressed by the splenic extract, and hence a greater pressor response ensues when adrenalin is injected than would otherwise be the case.

As such a heterogeneous group of tissues has been found to contain substances which act in a similar manner in antagonizing the inhibitory action of adrenalin upon certain uteri, and to a lesser degree depressing the cardiac vagus of the terrapin, it is possible that all tissues contain these principles in certain amount. It would in any case seem clear that these particular substances should not be regarded as "hormones."

SUMMARY

1. Extracts of various tissues were found to have definite stimulatory effect upon isolated intestinal and uterine musculature.

2. The primary effect of pancreatic extracts is depression in the tonus of the duodenal musculature.

3. The primary effect of extracts of tissue dried at 110°C. to 120°C. on the intestine is inhibition. This is followed immediately by definite stimulation.

4. Extracts of heart, spleen, pancreas, testes, anterior and posterior lobe of the pituitary body and thymus, thyroid and parathyroid glands in addition to stimulating the uterus of the rat, guinea pig, virgin dog and cat, antagonized the inhibitory action of adrenalin on these organs.

5. Extracts of heart and spleen caused the complete abolition of the response of the cardiac vagus to electrical stimulation.

6. The action of pilocarpine on the heart of the terrapin was partially but not completely antagonized by extracts of heart and spleen.

7. The substance or substances which antagonize the inhibitory action of adrenalin on the uterus and depress the cardiac vagus are soluble in alcohol and resist boiling.

8. Intravenous injection of extract of spleen augments the pressor response to adrenalin.

9. Intravenous injection of extract of heart raises the threshold for vagus inhibition by electrical stimulation of the peripheral end of the cut vagus.

10. It is suggested that substances similar to those found in extracts of various tissues may be present in greater or less amount in all tissues.

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RECIPROCAL REACTIONS IN THE CARDIO-VASCULAR SYSTEM

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INTRODUCTION

Sherrington (1) in his *Integrative Action of the Nervous System*, has enunciated the doctrine of reciprocal nervous relations existing in the living organism. Isolated facts pertaining to any biological mechanism or to any of its parts are valuable and useful if we can weave from them the whole fabric of truth. But we cannot escape the fact that functional systems, even in lower forms, are closely interrelated and interdependent. As Burdon-Sanderson has expressed it, the processes in the organism go on in the interest of the organism. In man the most complex of willed movements are possible because of the reciprocal innervation of many parts and systems, together with a central seat of integration for his sensory and motor mechanisms. Intake of food, digestion, metabolism and elimination, important as they are, cannot avail much if the blood supply to a part is interfered with by mechanical means, by the blocking out of nervous impulses experimentally, or by accident or disease.

One might expect to find a reciprocal functional relationship between the constituent parts of the cardio-vascular system. This research was undertaken with the object of arriving at a clearer understanding of the reciprocal relationships, whose existence one might be led to suspect, between the cardiac and the vascular nervous mechanisms, through experimental procedures which involved changes in the rate of the heart beat and the magnitude of arterial blood pressure.

From a simple functioning mechanism possibly, in some forms or at some stage of development, independent of nervous connections, the heart has been gradually developed phylogenetically into a complex differentiated organ. Throughout its history its activity has been dependent upon and modified by its increasingly complex physico-chemi-

cal environment. At first apparently devoid of nervous relations, later it appeared with nerve fibers connecting it with a more or less simple central nervous system, and it became subject to nervous control. As the central nervous system became more and more complex and highly organized in some of its levels, but less highly organized in others, the nervous connections of the heart multiplied the number of impulses modifying the inherent quality of rhythmicity possessed by the cardiac muscle tissue.

In the course of this development the automatism, originally common to all cardiac muscle, gradually disappeared from some parts, notably the apex. In the higher mammals, therefore, we find a heart with its automatic rhythmicity more and more under the influence of an increasingly complex physico-chemical environment and dependent for its normal activity upon nerve impulses received from the central nervous system.

In the normal animal the rate of the heart beat is closely connected with the blood pressure. If there is a large vasoconstriction, as in cold weather, the accompanying rise of blood pressure is associated with a slowing of the heart beat. Conversely, a vascular relaxation and lower blood pressure are accompanied by acceleration of the heart beat. From observation of these and other related facts Marey (2), in 1860, stated that the heart beat varies inversely as the blood pressure. In the slowing of the heart beat the vagus nerves are of course active, although in certain conditions, for example in muscular exercise, the increased blood pressure is not accompanied by a slower heart rate. It is hoped that the results of experiments recorded in this paper may give some information regarding the nervous mechanism for the adjustment of heart rate to changing blood pressure.

METHOD

The experiments were done on cats. The routine experimental procedure was ether anesthesia, tracheotomy and connection with a mercury manometer by means of a cannula placed in the left carotid artery. Blood-pressure and time tracings were made on a Hürthle kymograph and the heart rate was computed in beats per minute. The stage of anesthesia was determined principally by the presence or absence of the corneal reflex, light anesthesia, however, being always sufficient to abolish any sense of pain. In seeking some way of bringing about changes in blood pressure that could be readily measured without pro-

ducing any permanent lesions in the blood vessels and with these changes in pressure the consequent or accompanying changes in heart rate, it was found that digital compression of the abdominal aorta was a convenient method. Compression of the abdominal aorta was made just below the diaphragm and yet high enough to avoid any effect by pressure upon the splanchnic nerves. This was accomplished by placing the fingers under the vertebral column, the animal being in the back-downward position on the board, and sliding the thumb in just below the last pair of ribs and dorsal to the viscera until the groove on the ventral surface of the vertebral column is felt. The abdominal aorta lies in this groove, and can be compressed by pressure with the thumb. An alternative method is to slide the second and third fingers in from the side and compress the aorta by pressure with their tips. In order to be sure that no part of the effect was due to pressure on the splanchnic nerves, control experiments were made with a ligature placed around the thoracic aorta by means of a large aneurysm needle passed between the twelfth and thirteenth pairs of ribs. Results of compression of the aorta by means of this ligature coincided with those by means of digital compression. Compression of the descending aorta increases the volume of blood in the anterior portion of the cardio-vascular system and thus raises blood pressure and also increases pressure and velocity of blood flow in the coronary circuit and in the bulbar nerve centers.

Some of the experiments were made with the extrinsic cardiac nervous mechanism intact; others with this mechanism interfered with by certain experimental lesions. These lesions consisted in severing the vagi, removing the stellate ganglia, thus abolishing the influence of the accelerator mechanism, dividing the dorsal roots of the spinal nerves, and dividing both dorsal and ventral roots of the thoracic nerves. Some experiments were also made on the relation of heart rate and blood pressure to hemorrhage. Details of the various types of experiments are given under the respective headings.

RESPONSE OF HEART RATE AND BLOOD PRESSURE TO COMPRESSION OF THE ABDOMINAL AORTA

1. With cardio-vascular nervous mechanism intact. It was desired first to determine the nature of the response of heart rate to changing blood pressures with the cardio-vascular nervous mechanism intact. This was done by compressing the abdominal aorta when the animal was under different stages of ether anesthesia, and also when there had been

interference with the blood supply to the bulbar nerve centers. The data are presented in table 1.

By reference to table 1 it is seen that under light anesthesia compression of the abdominal aorta causes the carotid blood pressure to rise immediately; during the continuance of the compression there is a gradual but marked fall in carotid pressure until the aorta is released. Then the pressure falls immediately to a point below its initial level. Subsequently the pressure continues to fall, but soon rises to the level before compression. The high carotid pressure produced by compression of the aorta is accompanied by a marked decrease in heart rate. When the aorta is released and pressure falls the heart rate increases, but does not immediately reach the initial rate. From the averages of

TABLE 1

Response of heart rate and blood pressure to compression of the abdominal aorta, with cardio-vascular nervous mechanism intact. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery

CONDITION	NO. OF EXPERIMENTS	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
		Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
Light anesthesia	50	206	124	178	197–166	193	110–76–108
Deep anesthesia	25	197	101	192	164–155	191	102–82–87
Restricted blood supply	23	161	56	158	103–97–100	147	55–46–43
Reversed reaction	7	149	107	164	145–149–140	182	103–84–101

fifty compressions upon thirty-five different animals under light anesthesia, there was thus a compensatory response of heart rate to changing blood pressures and Marey's law of inverse ratio of heart rate and blood pressure applies.

Under anesthesia deep enough to abolish the corneal reflex the initial blood pressure and heart rate are lower than under light anesthesia. Compression of the aorta does not raise the carotid pressure as high as under light anesthesia and there is not the marked fall in carotid pressure during continuance of the compression. When the aorta is released carotid pressure falls immediately to about the initial level. Subsequently it falls more gradually than under light anesthesia, then rises very slowly. The high carotid pressure produced by compressing

the aorta is usually accompanied by a very slight decrease in heart rate, but this decrease is wanting in very deep anesthesia. When the aorta is released and pressure falls the rate remains about the same or decreases slightly. From averages of twenty-five compressions on animals under deep anesthesia, it is thus seen that there is usually a slight compensatory response of heart rate to blood pressure when the pressure is high in the medulla oblongata; when the pressure is low, as upon release of the aorta, this compensatory mechanism is seriously interfered with and the return to the initial rate and pressure is either permanently lost or very much delayed.

When the blood supply to the medulla oblongata is restricted, as in hemorrhage or in some instances of partial ligation of the head arteries, blood pressure falls and heart rate decreases. Now when the abdominal aorta is compressed the carotid blood pressure rises immediately, as in light anesthesia, but not to the same extent. During the continuance of the compression, blood pressure at first falls and then rises slightly. When the aorta is released the carotid pressure immediately falls to about the initial level, and then continues gradually downward. When carotid pressure is high during compression of the aorta, the heart rate decreases somewhat, but when the aorta is released and carotid pressure falls the heart rate decreases with the falling pressure. If the anemia of the medulla be severe or long continued, the return to the initial heart rate and blood pressure is lost.

Under certain conditions there appears what may be termed a "reversed reaction." This is sometimes observed if the abdominal aorta is compressed: *a*, during the early period of recovery following excessively deep anesthesia; *b*, during recovery from pronounced asphyxia; *c*, shortly after the reëstablishment of the blood supply to the medulla oblongata after hemorrhage; and *d*, during restricted blood supply from ligation of the head arteries. Instead of a compensatory slowing of heart rate in response to the increased blood pressure, the rate increases during the period of high pressure and continues to increase even after release of the aorta and consequent fall of pressure.

After observing the occurrence of this so-called "reversed reaction" many times in the earlier experiments of this research and noting that it appeared under the conditions above mentioned, it was decided to try to reproduce this type of response under controlled conditions. By referring to reactions 15 and 16 in the protocol of December 23, 1919, (p. 360) it will be seen that this duplication was possible. This reaction follows the recovery of the bulbar centers from anemia produced

Protocol of experiment of December 23, 1919. Ether; tracheotomy. Blood pressure cannula in left carotid artery. Ligature around abdominal aorta between twelfth and thirteenth pairs of ribs. Stage of anesthesia denoted by presence or absence of corneal reflex (c.r.+ or c.r.−). Experiment terminated by the hemorrhages. Sequence of events indicated in "Reaction No." columns.

Reaction number		Rate	Pressure	Reaction number	Before compression: Heart rate	Before compression: Blood pressure	Before compression: Anesthesia	During compression: Heart rate	During compression: Blood pressure	During compression: Anesthesia	During compression: Time	After compression: Heart rate	After compression: Blood pressure	
											seconds			
1	*11:35* After tracheotomy	186	126											
				2	186	126	c.r.+	160	200–146	c.r.+	19	168	92–84–118	
	Lighter anesthesia than above			3	176	112	c.r.+	461	188–146	c.r.+	19	188	98–72–118	
4	Before	186	126											
5	*11:52* After placing ligature around abdominal aorta	156	108											
				6	156	108	c.r.+	133	162–126	c.r.+	19	160	100–82–100	
				7	168	106	c.r.+	151	162–130	c.r.+	19	162	102–80–102	
	Rt. carotid compressed. Light anesthesia			8	162	112	c.r.+	150	174–146	c.r.+	20	159	102–86–104	
	Rt. carotid compressed. Deeper anesthesia			9	*155*	*100*	c.r.+	*157*	*146–140*	c.r.−	*27*	*154*	*104–82–100*	
10	Carotid released—pressure falls; respiration very shallow													
	Artificial respiration			11	212	80	c.r.−	192	148–134	c.r.−	15	189	100–80–90	
	12:01			12	198	106	c.r.−	181	160–186–164	c.r. return	38	200	104–64–94	
	Panting respiration (artificial respiration discontinued)			13	213	98	c.r.+	175	174–140	c.r.++	26	208	94–68–104	Cat restless
	Clamped right carotid and right vertebral			14	216	110	c.r.+	191	204–184	Cat restless	22	212	100–74–124	
					Released R. carotid and R. vertebral.		c.r.+	198	184–144–154		26			
	More ether; deeper anesthesia than 14. Reversed reaction			15	144	106	c.r.++	163	154–132–140	c.r.+	25	189	104–96–100	

	Partial asphyxiation between 15 and 16	16	174	96	c.r.+	177	136–144–126	c.r.+	20	192	96–78–88		
	Reversed reaction												
	12:14	17	195	108	c.r.+	175	176–144	c.r.++	25	180	100–84–102		
	12:30 Lighter anesthesia (ether out just before 18)	18	186	104	c.r.+	168	184–144–148	c.r.+	41	175	108–82–98		
		19	192	108	c.r.++	167 160 } 163	184–152 152–158	c.r.+	24 24	165	108–74–94		
20	*12:40* Before (ether intermitted)	186	c.r.+	120									
21	After hemorrhage of 35 cc.	168	c.r.+	52									
		22	168	52	c.r.++	160	92–118–124	c.r.+	37	156	84–48–54	c.r.+	
	12:44	23	165	60	c.r.+	155	136–124–144	c.r.++	57	135	84–46–56		
	12:49 Ether out; very light anesthesia	24	136	64	c.r.+	123	128–110–120	c.r.++	40	110	84–54–60		
	1:10 Recovery from deep anesthesia	25	192	62	c.r.−	160	138–98–104	c.r.+	53	170	74–40–60		
	1:15	26	170	68	c.r.++	168	142–120–112	c.r.+	50	148	56–36–60		
	1:25 After artificial respiration compression brings back normal respiration	27	189	56	c.r.−	171	84–100–84	c.r.−	46	158	50–32–50		
28	A further hemorrhage of 13 cc. (total of 48 cc.) produces respiratory gasps, a great fall of blood pressure, great slowing of heart and death at *1:54*												

by ligating the head arteries even when the anemia has been severe enough to necessitate artificial respiration. The first of these compressions (reaction 15) was made when the heart rate and blood pressure were falling; the second (reaction 16) shortly after and at a higher level of heart rate and blood pressure brought about by the increased pressure and velocity of blood flow in the medulla oblongata by the previous compression of the abdominal aorta.

The most salient points of the facts above mentioned, when the cardio-vascular nervous mechanism is intact, are: *a*, Under light anesthesia there is a compensatory response of heart rate to changing blood pressures. *b*, Under deep anesthesia and also when the blood supply to the medulla is severely restricted there is a slight compensatory response of rate to pressure when pressure is raised, but when pressure falls this response is completely abolished. *c*, There seems to be a certain level to which the functional activity of the bulbar cardio-vascular mechanism may sink before permanent injury is done to these central cells, a level at which subsequent increase of blood pressure and velocity of blood flow will bring back their function, a previously decreasing heart rate being accelerated and a blood pressure being restored equal to or nearly equal to that before the insult to the bulbar mechanisms occurred.

2. *Response of heart rate and blood pressure to compression of the abdominal aorta, with experimental lesions.* The behavior of the cardio-vascular mechanism to changes in blood pressure when experimental lesions have been produced by sectioning some or all of the extrinsic cardiac nerves is shown in tables 2 and 3. In these tables averages are given to show the type of response of heart rate to changes in blood pressure when the mechanism is intact, for comparison with the types of response when various lesions in the mechanism have been produced experimentally.

In those experiments in which section of the extrinsic cardiac nerves was ultimately desired, the routine procedure was ether anesthesia, tracheotomy, exposure of vagi and dissection for exposure of the stellate ganglia by Anderson's method as described in Sherrington's *Mammalian Physiology* (3).

It will be observed that changes in heart rate and blood pressure due to the operative procedures alone are not great. Marey's law of inverse ratio of rate to pressure holds upon compression of the abdominal aorta after dissection for the exposure of both the vagi and the stellate ganglia, but, in the case of the latter, at a slightly lower level of pressure and rate.

The specific differences in the types of response, when lesions have been produced experimentally in the extrinsic cardiac nerves, are here set forth.

a. Division of vagi, accelerators intact. If the abdominal aorta is compressed after bilateral vagotomy (table 2), when the accelerators are intact, the compensatory response of a lower heart rate to the increased blood pressure remains, but the degree of compensation is much less than before division of the nerves. The level of pressure is about the same as, and the rate slightly higher than, before section of the vagi. When the aorta is released and pressure falls, the compensatory response of a higher rate to a falling pressure is lost and a small decrease in rate accompanies the fall of pressure.

TABLE 2

Response of heart rate and blood pressure to compression of the abdominal aorta, after certain experimental lesions. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery. Averages from experiments of November 12, 14, 19 and 26, 1919

CONDITIONS IN SEQUENCE OF OCCURRENCE	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
After tracheotomy...	223	127	183	202–178	214	104–130
After dissection for stellate ganglia....	184	102	166	173–159	171	99–66–81
After bilateral vagotomy	187	99	181	171–168	178	103–75–76
After excision of stellate ganglia....	161	78	156	135–133	149	87–61–68

b. Division of vagi followed by excision of accelerators. If the abdominal aorta is compressed after excision of the stellate ganglia, subsequent to division of the vagi, there remains the compensatory response of a decreasing heart rate to an increased blood pressure. Both rate and pressure are at a lower level than when the stellate ganglia are intact, the level of pressure being comparatively lower than that of rate. When the aorta is released and blood pressure falls the heart rate does not increase but decreases with the falling pressure.

c. Excision of accelerators followed by division of vagi. The deportment under the conditions *a* and *b* may be contrasted with what occurs when the accelerators are removed first (table 3). Here, immediately

after removal of the ganglia, the pressure is found to have fallen greatly and the rate to have decreased somewhat. During compression of the abdominal aorta the cardio-vascular mechanism, after the first sudden rise of pressure, compensates, not by the usual gradual fall, but by a gradual rise of pressure which continues during the whole time of compression; compensation of rate to pressure is greatly diminished, this compensation being entirely lost after release of the aorta and the resulting fall of pressure. Bilateral vagotomy itself, after the accelerators are removed, causes a further small fall in blood pressure but an increase in heart rate. Compression of the abdominal aorta now brings the blood pressure higher, with the same inability on the part of the

TABLE 3

Response of heart rate and blood pressure to compression of the abdominal aorta, after certain experimental lesions. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery. Averages from experiments of October 24, 28 and 29, 1919

CONDITIONS IN SEQUENCE OF OCCURRENCE	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
After tracheotomy...	238	155	171	215–184	212	135–112–137
After dissection for stellate ganglia....	198	115	182	181–156	190	105–75–106
After excision of stellate ganglia.....	167	63	164	81–91	162	67–53–49
After bilateral vagotomy.....	178	52	177	79–101	170	79–61–49

cardio-vascular mechanism to bring about a fall of pressure during the maintenance of compression. Instead, the blood pressure continues to rise and, when the aorta is released, falls more gradually than before. The compensation of rate to pressure is almost lost during compression and entirely lost when the pressure falls after release.

It is thus seen that when all the extrinsic cardiac nerves are severed either the heart rate falls somewhat and blood pressure considerably, or there may be a greater fall in heart rate and only a moderate fall in blood pressure. The final result seems to depend on whether the vagi or the accelerators are sectioned first. After division of the vagi, when the accelerators are intact, the heart rate usually increases somewhat though the blood pressure may remain the same or may fall slightly.

While there is not sufficient increase in rate to influence pressure, the maintenance of pressure seems in some way to be connected with the accelerators (see Hunt, 4), as is shown by what happens when the stellate ganglia are subsequently removed, for then both the rate and pressure fall.

Removal of the accelerators alone results in a moderate decrease in heart rate and a great fall in blood pressure. Now when the vagi are divided subsequent to excision of the stellate ganglia and the impulses inhibiting the heart rate are removed, the rate increases somewhat though it does not reach the rate it maintained before the accelerators were removed, while the pressure continues to fall. After an interval the latter sometimes rises, which rise may possibly be due to the removal of the "tonic" effect of the cardiac nerves over the heart musculature (5), (6), (7), and a greater volume of blood entering the heart.

The following summary will serve to make more clear the above statements:

Averages from experiments of November 12, 14 and 26, 1919

	Rate	*Pressure*
Before vagi are sectioned	183	98
After vagi are divided	188	96-98
After stellate ganglia are removed	161	80
Percentage fall when vagi are divided first	12	19·2

Averages from experiments of October 24, 28 and 29, 1919

	Rate	*Pressure*
Before stellate ganglia are removed	195	116
After stellates are removed	167	63
After vagi are divided	178	51
Percentage fall when stellate ganglia are removed first	8·7	55

We might add that when the extrinsic cardiac nerves are divided the type of response to compression of the abdominal aorta also seems to depend on whether the vagi or accelerators are divided first, as we have seen above (*a*, *b* and *c*).

With all the extrinsic cardiac nerves cut the heart rate is not so susceptible to the effects of low blood pressure as when the nerves are intact. This is shown by observations made after the injection of sodium nitrite and after hemorrhage. In both of these cases the heart rate is relatively high even when blood pressure is approaching the base line. This is to be contrasted with the gradual simultaneous fading-out of rate and pressure when the nerves are intact.

In this research it has been observed also that the left ventricle is not firmly contracted after section of the cardiac nerves. This is in accordance with previous observations of other structures. It has been found by Bierfreund (cited by Pike, 8) that the onset of post-mortem rigidity is delayed by the hemisection of the spinal cord. This occurs on the hemisected side. Sherrington also found later that the onset of rigor mortis in the hamstring muscles is delayed by section of the appropriate dorsal spinal nerve roots of the same side.

TABLE 4

Response of heart rate and blood pressure to compression of the abdominal aorta, after certain experimental lesions. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery. Averages from experiments of June 6, 11, 16 and 17, 1919

CONDITIONS IN SEQUENCE OF OCCURRENCE	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
After tracheotomy and laminectomy	240	108				
After section of dorsal spinal roots	230	85	196	152–142	200	72–61
After bilateral vagotomy	222	58	214	121–114	201	59–52
After removal of stellate ganglia	187	34	183	71–82–81	165	35–26
After section of dorsal roots						
Light anesthesia	223	78	189	155–134	201	88–64–61
Deep anesthesia	191	58	191	112–101	186	55–47

d. Section of dorsal roots of spinal nerves. The data obtained on the response of the cardio-vascular mechanism to changes in blood pressure after section of the dorsal spinal roots in a region as extensive as cervical 6–7 to lumbar 1–2 are presented in table 4.

Section of the dorsal spinal roots in this region does not abolish the compensation of heart rate to high and low blood pressures. The law of inverse ratio of rate to pressure still holds true and to practically the same extent during compression of the aorta and the resulting increased pressure. This is shown also by the following brief summary from the data of tables 1 and 4.

During compression of the abdominal aorta and high blood pressure.
After tracheotomy, decrease in heart rate = 13.6 per cent.
After laminectomy, decrease in heart rate = 15.8 per cent.
After section of dorsal roots, decrease in heart rate = 14.7 per cent.

When the aorta is released and pressure falls, the degree of increased heart rate to the falling pressure is less after section of the dorsal roots than before:

After release of the abdominal aorta and falling blood pressure.
Before section of dorsal roots, increase in heart rate = 7.7 per cent.
After section of dorsal roots, increase in heart rate = 2.0 per cent.

The similarity of response after section of the cardiac nerves before and after section of the dorsal roots is to be noted. Bilateral vagotomy after section of the dorsal roots produces a somewhat lower level of pressure than it does when these roots are intact. Removal of the stellates, subsequent to bilateral vagotomy after section of the dorsal roots, results in a much lower level of rate and pressure than occurs when these cardiac nerves are divided before the dorsal roots are cut.

Previously it has been noted that after section of the extrinsic cardiac nerves compensation of rate to pressure holds to some extent during high pressure, as during compression of the abdominal aorta, but is lost after release of the aorta and a falling pressure. This is also true when the extrinsic cardiac nerves are divided after section of the dorsal roots, although the fall in rate after release of the aorta is greater than when the dorsal roots are intact. Sherrington (9) notes the general maintenance of arterial pressure after section of the dorsal spinal roots in the thoracic region.

There seems to be a greater sensitivity of the cardio-vascular mechanism to deep anesthesia after the dorsal roots are sectioned than before. This is shown not only by the complete lack of compensation of rate to pressure when the abdominal aorta is compressed, but by both rate and pressure falling to a much lower level than when the roots are intact.

e. Section of dorsal and ventral roots of thoracic spinal nerves followed by stimulation of sciatic nerve. The usual response of an increased heart rate and blood pressure to stimulation of the sciatic nerve is shown in figure 1 and table 5. Here it is also seen that this familiar response to sciatic stimulation seems to be abolished after section of the spinal nerve roots, thoracic 1–5, independently of whether weak or strong stimuli are used.

It is impossible to say, on the basis of the number of experiments done, whether the above lack of response to sciatic stimulation is a necessary effect of section of these roots, or whether it is due to some error of technique which has not yet been detected. Section of these roots, which lie in the region of the emergence of the cardiac accelerators does not, however, abolish the compensatory ratio of rate to pressure upon compression of the abdominal aorta but does alter the type of response of the cardio-vascular mechanism to changes in pressure from that seen when the accelerators alone are removed. Instead of a steady climb of pressure upward, when the accelerators only are removed, after root section pressure first rises and then steadily falls. And there remains a slight compensation of an increased rate to the falling pressure

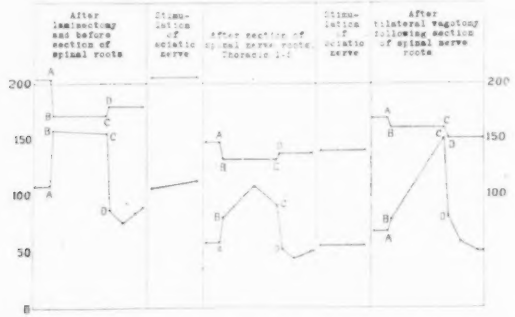

Fig. 1. Response of heart rate and blood pressure to stimulation of the sciatic nerve and to compression of the abdominal aorta, after certain experimental lesions. Lower curves represent the pressure in the carotid artery; figures at the left indicate actual pressures in mm. Hg. Upper curves represent the rate of the heart; figures at the right indicate rates per minute. *A* = point of compression of abdominal aorta; *C* = point of release of aorta.

when the aorta is released, which is totally abolished when the stellates only are excised. An increased rate follows bilateral vagotomy after section of these spinal roots as it does when the vagi are sectioned after removal of the stellates. But when the aorta is compressed, after root section and bilateral vagotomy, the pressure rises steadily and much higher than when the cardiac nerves alone are cut, and the heart rate falls with the falling pressure after release of the aorta.

Further investigation of the results of section of these spinal roots in combination with lesions in other parts of the cardio-vascular mechanism will probably prove of much interest.

TABLE 5

Response of heart rate and blood pressure to stimulation of the sciatic nerve and to compression of the abdominal aorta, after certain experimental lesions. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery

CONDITIONS IN SEQUENCE OF OCCURRENCE	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
After laminectomy	203	107	170	157–155	179	86–75–83
Stimulation of sciatic nerve	205	107–113				
After section of spinal nerves, thoracic 1–5	147	58	131	80–109–91	136	53–45–50
Stimulation of sciatic nerve	No effect					
After lateral vagotomy	169	67	160	82–117–152	150	80–58–50

RESPONSE OF HEART RATE AND BLOOD PRESSURE TO HEMORRHAGE

The rôle played by the accelerators is well shown in one particular group of experiments, for when severe hemorrhage is followed by division of the vagi there is a conspicuous increase in heart rate, though not enough to rescue the slightly falling pressure. *The accelerators undoubtedly respond to the emergency of a falling blood pressure.* The summary below presents the data of three experiments.

		HEART RATE	BLOOD PRESSURE
February 10, 1919.	Before hemorrhage	183	150
	After hemorrhage	156	56
	After bilateral vagotomy	160	60
February 17, 1919.	Before hemorrhage	163	162
	After hemorrhage	156	54
	After bilateral vagotomy	204	56
February 21, 1919.	Before hemorrhage	148	120
	After hemorrhage	125	34
	After bilateral vagotomy	141	22
Averages:	Before hemorrhage	165	144
	After hemorrhage	146	48
	After bilateral vagotomy	168	46

The rôle of the accelerators is further elucidated by an examination of the data obtained when hemorrhage follows excision of the accelerators on one or both sides with or without bilateral vagotomy (table 6).

The above table presents some interesting comparisons. A hemorrhage of sufficient volume to cause a great fall in blood pressure before a break in the heart rate occurs, when the cardio-vascular mechanism is intact (1), causes a somewhat greater fall in pressure and nearly a 50 per cent decrease in rate when the accelerators are excised (2). With

TABLE 6

Response of heart rate and blood pressure to hemorrhage in connection with certain experimental lesions in extrinsic cardiac nerves. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery

CONDITION	HEART RATE		BLOOD PRESSURE	
	Before hemorrhage	After hemorrhage	Before hemorrhage	After hemorrhage
1. Experiment April 1, 1919.				
Cardiac nerves intact	180	180	100	24
Percentage change		0		75
2. Experiment December 9, 1919				
Accelerators excised (vagi intact)	158	90	80	16
Percentage change		43		80
3. Experiments October 29 and December 17, 1919				
Cardiac nerves excised (accelerators out first + bilateral vagotomy)	160	150	71	16
Percentage change		6.2		77.4
4. Experiment December 13, 1919				
Excision of left stellate; bilateral vagotomy (right stellate intact)	216	210	126	28
Percentage change		1.8		77.7

all the extrinsic cardiac nerves severed (3), the fall in pressure is nearly as great but with only a slightly lower rate (6.2 per cent decrease) than before hemorrhage occurred. With the accelerators of one side intact (4) the fall in pressure is about the same as when all the cardiac nerves are severed but the decrease in rate is somewhat less (1.8 per cent). In the last two instances it is again seen that the rate is not so susceptible to low pressure when all the extrinsic cardiac nerves are cut.

Various writers have called attention to the fact that the difference in response to stimulation of the accelerators depends principally on the

frequency of rhythm before excitation. It seems also that this difference in response depends upon the level of blood pressure during excitation. This is shown by results obtained when the accelerators are stimulated during periods of a diminishing blood volume produced by a series of hemorrhages. When the pressure is low, but the rate yet high, it is found that there is not as great an increase in rate from stimulation as when the pressure has fallen lower from a greater loss of blood and the rate too begins to decrease. It would thus appear that there is a level of pressure at which the effect upon rate from stimulation of the accelerators is greatest, other factors being equal. It may also be said that the accelerators respond to artificial stimulation when the blood pressure has fallen much below this level of greatest response to excitation.

DISCUSSION

In the beginning of this paper it was stated that this work was undertaken with the object of arriving at an understanding of the reciprocal functional relationships existing between the cardiac and vascular nervous mechanisms. That this relationship exists can hardly be doubted when we remember that both heart and blood vessels have their own particular functionally antagonistic innervations. To the heart go efferent fibers that carry impulses from the central nervous system which increase or diminish its rate, and to the blood vessels go efferent fibers that bring about changes in blood pressure by dilating or contracting their vascular walls. The nervous centers specifically concerned with the regulation of this cardio-vascular mechanism, located in the region of the medulla oblongata, have a wide relation with afferent nerve fibers from all parts of the body, including the heart itself. This makes it possible for the heart and blood vessels to be directly or reflexly influenced in their behavior. Changes in rate and amplitude of the heart beat, differences in volume and tone of the heart musculature, and changes in the peripheral resistance may all be brought about by nervous impulses or by changes in the character of the blood flow through the medulla oblongata. With this in mind and remembering that heart rate is closely related to blood pressure and that conditions which affect one influence the other, we can now consider the significance of the differences shown in the behavior of this mechanism when various parts of its regulatory system have been abolished.

The differences, we may say at the outset, are closely related to conditions in the bulbar mechanism. When it is interfered with by partial

or complete anemia, asphyxia or too deep anesthesia, the effect on rate and pressure following section of the cardiac nerves is not as great as when the blood supply to the medulla is normal. And we can say also, from observation of many experiments, that the vasomotor center seems more susceptible to such changing conditions when part of the nervous mechanism of the cardio-vascular system is eliminated. When bulbar conditions are favorable there is a reciprocal response on the part of the vasomotors, and blood pressure gradually rises after its first fall following section of the cardiac nerves. If the accelerator branches from the stellate ganglia have been sectioned this rise of pressure is accompanied by a marked increase in heart rate, which is possibly, even probably, due to accelerator impulses that reach the heart through the cervical ganglia (10), (11). But if, instead of a mere section of the accelerator branches, the stellate ganglia have been removed this rise of pressure is unaccompanied by a change in rate. In many other cases of a steadily falling pressure and presumable failure of the vasomotors, pressure can be raised by manipulation of the lower extremities and abdomen and a redistribution of the blood.

The ultimate heart rate and blood pressure after section of the cardiac nerves seems to depend on whether the accelerators or vagi are divided first. In each case the partially intact mechanism apparently adjusts itself to the altered conditions, but the manner of adjustment differs with the sequence of section. With the accelerators removed and the vagi intact, the rise of pressure during manual compression of the aorta is not as great as when the impulses coming over the vagi are also blocked out. It is as if this inhibitory mechanism were standing guard against a rising tide of pressure that might do violence to the best interests of the organism. When the vagi are divided, with the accelerators intact, pressure from compression of the aorta rises higher than when the accelerators are excised. The fall in pressure, after release of the aorta, continues longer and is greater than before excision of the accelerators. With the accelerators removed it is as if the cardio-vascular mechanism had lost part of its efficient protection against a falling blood pressure and the resultant harm to the multitude of tissue cells.

These results would lead one to the conclusion that when an increased heart rate occurs in the organism through the mediation of the cardio-vascular nervous system, it is due to a positive activity on the part of an accelerating mechanism and not to some mechanism that inhibits the activity of the inhibitors.

This idea of a mechanism of inhibition of inhibition for the regulation of heart rate and blood pressure in the cardio-vascular system may be said to begin with the discovery in 1845, by the Weber brothers, that stimulation of the vagi inhibits the heart rate. E. H. Weber extended the notion of inhibition to the increased spinal activity after ablation of the brain. Following the Webers came Setschenow, who found that stimulation of the midbrain and bulb prolongs reflex time and also that a frog draws its foot from acidulated water much later if the brain be stimulated. But it was not until after his work, in 1863, that "this notion of the inhibition of activity of one part of the central nervous by the activity of another became a working physiological thesis" (12). Later it was shown by L. N. Simonoff (1881) and by A. Herzen (1884) that inhibition of spinal reflexes was obtained from other foci in the brain and cord itself. This was done by sensory stimulation or by direct stimulation of the foci themselves. "But," as Pike has said, "in all this infatuation with the hypothesis of inhibition, its devotees lost sight of the accompanying positive motor phenomena. Until some reason is advanced for this oversight, the hypothesis of inhibition is open to suspicion of being overworked."

In the light of later work on the general life processes of biological mechanisms, it seems that Setschenow's interpretation took color, as so often happens, from the depressing political and economic conditions of his time. If the degree of activity of the inhibiting center is itself dependent upon inhibition, and if the processes in the organism go on in the interests of the organism, as Burdon-Sanderson phrased it, why is there not then this activity of inhibition of inhibition when the accelerators are removed so that the heart rate and blood pressure may be kept up? The activity of the inhibiting vagi, however, is not stopped until the impulses coming over the vagi have been blocked out by double vagotomy or by atropine, or if it is stopped, the results are not of the same magnitude as when the accelerators are present.

It seems rather difficult to apply an hypothesis of inhibition of inhibition for the regulation of heart rate and blood pressure in the cardio-vascular mechanism in view of results obtained in this series of experiments and the facts just stated.

My results however, do, I believe, provide sufficient evidence to postulate a simpler mode of action of the inhibitor-accelerator nervous mechanism. This nervous mechanism consists of: *a*, An inhibitor mechanism acting positively in the best interests of the organism; i.e., to prevent overwork by a too rapid heart rate and to bring about suf-

ficient periods of rest for metabolic repair of the heart musculature. The exercise of this function of the mechanism is not regularly mediated by means of inhibitory influences acting from without upon the mechanism itself; *b*, an accelerator mechanism acting positively in the best interests of the organism and intimately connected with the maintenance of blood pressure. These two functionally different parts of this mechanism possess different thresholds of sensitivity to the same forms of stimuli and varying degrees of sensitivity to each of the various physico-chemical changes occurring within the organism.

Such an hypothesis is in complete accord with the physiological and pharmacological antagonism of other systems innervated, as is this cardio-vascular system, through the cranio-sacral and thoracico-lumbar divisions of the autonomic nervous system.

One further point comes out with regard to the organization and general deportment of the cardio-vascular nervous mechanism, suggested by Prof. F. H. Pike.

The degree of organization, to use Hughlings Jackson's expression, of the neural portion of the cardio-vascular mechanism is high; the responses are, in general, very specific in character, and marked by the absence of any great variety of combinations. Marey's law expresses a definite and relatively unvarying relationship existing between two very definite conditions. The result may be interpreted as being in the interests of the organism. The organization of the central mechanism is not, however, so rigid as to prevent dissociation of these two reciprocal responses of heart rate and blood pressure when the conditions are such that it is in the interest of the organism for this dissociation to occur. As an illustration of this we may cite the deportment of the cardio-vascular mechanism when the blood supply to the medullary portion of it is reduced. If the central mechanism demands a certain definite set of conditions in the way of volume of blood flow and blood pressure for the maintenance of its function, it is obvious that such conditions can be kept uniform only if the whole mechanism has certain powers of adaptation to changing conditions. The adaptation expressed in the sequence of events described by Marey's law is an adaptation to one set of conditions. If conditions should change in such a way as to demand a *high* systemic blood pressure for the fulfillment of the requirements of the central mechanism in the way of volume of blood flow and blood pressure, a falling heart rate in response to such a high systemic blood pressure would be distinctly *not* in the interest of the organism. Haldane has suggested that the rigidity of the deport-

ment of the respiratory mechanism may, under certain conditions, act contrary to the interest of the organism. On that view, the dissociation of the normal response to high systemic blood pressure is to be regarded as an adaptation to restriction of the volume of blood flow through the medulla. It would appear that in the case of the cardio-vascular mechanism as well as in the case of the respiratory mechanism, it is the conditions in the central mechanism itself which, in part at least, determine its degree and state of activity (13).

This dissociation of the usual reactions of the cardio-vascular mechanism is analogous to the dissociation of responses in vertigo, as pointed out by Wilson and Pike.

To Prof. F. H. Pike I offer grateful acknowledgment.

SUMMARY

1. Marey's law of inverse ratio of rate and pressure holds in light anesthesia (deep enough, however, to insure complete anesthesia).

2. This compensation of rate and pressure is lost or seriously interfered with in deep anesthesia; also, in restricted blood supply to the medulla.

3. What may be termed a "reversed reaction" appears under certain conditions of anemia of the bulbar mechanism. There is dissociation of the usual reactions of the cardio-vascular mechanism.

4. The accelerators undoubtedly respond to the emergency of a falling blood pressure.

5. When the accelerators are excised there is a greater percentage change in rate and pressure both falling lower after hemorrhage than when the accelerators and vagi are cut.

6. These experiments demonstrate the tonic activity of the accelerators.

7. The ultimate heart rate and blood pressure, after section of the extrinsic cardiac nerves, depends on whether the vagi or accelerators are excised first.

8. Section of the dorsal spinal roots, cervical 6–7 to lumbar 1–2, does not abolish the compensatory response of heart rate to high and low blood pressures.

9. When the extrinsic cardiac nerves are divided the musculature of the left ventricle remains more flaccid after death than when the cardiac nerves are intact.

10. There is an inhibitor-accelerator nervous mechanism acting positively for the control of the cardio-vascular system.

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FURTHER OBSERVATIONS ON THE RELATION OF INITIAL LENGTH AND INITIAL TENSION OF AURICULAR FIBER ON MYO- AND CARDIODYNAMICS

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INTRODUCTION

In a previous paper (1) the relation of initial length and initial tension of auricular muscle to strength of muscular contraction was pointed out in a qualitative way. The present paper represents a more exact and quantitative study of the same problem.

Blix (2) demonstrated in the case of striated muscle of the frog that tension developed is a linear function of initial length of muscle fiber. Evans and Hill (3), employing the same muscle, find heat and tension developed to increase in direct proportion to the increase in length of muscle through an extension of the original unloaded length amounting to approximately 15 per cent. These changes in length, according to Evans and Hill, approximate those normally occurring in situ.

The auricle of the turtle may undergo far greater changes in length of fiber and consequently is a suitable structure not only for determining whether cardiac muscle follows the same laws as striated muscle but also whether the linear function of tension developed holds for these great changes in length of fiber.

It was demonstrated (1) that an increased auricular volume was accompanied by a striking increase in strength of contraction. Though the results indicated at that time that length of fiber was by far the most important factor influencing the strength of contraction yet some results were obtained suggesting that initial tension might play a minor rôle in determining the force of contraction. Consequently methods were used to determine the relative importance of both initial length and initial tension of the cardiac muscle.

METHODS

It is realized that the parallel fibered muscle offers the best condition for studying the strength of muscular contraction; that the interlacing fibers and the reticular arrangement of the bundles of fibers in the auricles may offer difficulties. These difficulties are largely met by recording the pressures developed within the intact auricle rather than the tension developed by a strip of auricular tissue. The method necessarily introduces factors involved in the mechanics of the hollow sphere and the results therefore have an additional interest in supplementing earlier papers on the relation of cardiac volume to cardiodynamics (1), (5), (6) and (7).

The methods employed are similar to those previously described (1). The auricle suspended in saline solution in a stoppered vessel is connected with a membrane manometer and a reservoir also containing saline solution. A simple maximum, minimum and mean valve devised for these experiments is interposed between the auricle and the reservoir to permit the recording of isometric contractions with progressively changing auricular volume (initial length). The changes in auricular volume are recorded with a piston recorder in connection with the air space above the saline solution in which the auricle is supsended.

In the records shown, the upper are the volume tracings, the lower are the tension tracings. Upstrokes represent respectively an increase in volume and increase in tension; downstrokes, the reverse.

In these experiments we are interested not only in the amount of pressure developed by the entire auricular musculature which is recorded directly with the membrane manometer but in the actual amount of tension supported per unit of muscle as well.. To determine this we must take into account the changing auricular surface for the same amount of musculature spreads over a changing surface all of which is supporting the recorded pressure. Assuming the auricle to be spherical (it is of course only roughly so) and knowing its volume, the inner auricular surface can be computed. Multiplying this by the recorded initial and final pressure we get the tension per unit of muscle.

RESULTS

The data of this research were obtained from twenty-two experiments on the auricles of nine turtles (Pseudemis elegans). Tables are published for three of these experiments accompanied in two instances by the records from which the data were obtained. The data are tabu-

lated in the following way (see table 1): Column 1—number of contraction; columns 2, 3 and 4—initial pressure, final pressure and pressure developed or the difference between initial and final pressure—all in millimeters of water as recorded by the manometer (these are the intra-auricular pressures sustained and developed disregarding the changing inner auricular surface); columns 5, 6 and 7 represent auricular volume, inner auricular surface and length of auricular fiber in cubic, square and linear millimeters respectively; columns 8, 9 and 10 the tension sustained and developed taking into account the changing auricular surface. These are given in weight (in cubic millimeters of water) sustained by the total inner auricular surface.

Of this data final tension and tension developed are plotted on the ordinates against length of fiber on the abscissas, in figure 1, *a*, *b*, *c* and *d*. An idea of the changes in initial tension sustained is obtained by noting the vertical distance between the curves of final tension and tension developed. In figures 4, *a*, *b*, *c*, *d* and *e* the curves of initial tension are plotted as well.

THE RELATION OF INITIAL LENGTH OF FIBER TO STRENGTH OF MUSCULAR CONTRACTION

Previous results (1) on the auricle of the turtle pointed to initial length of fiber as the important factor determining the amount of tension developed agreeing with the conclusions of Patterson, Piper and Starling (9) on their work on the mammalian ventricle. My conclusions were reached from records similar to figure 3 of the present paper. It will be noted in this record that the strength of contraction progressively increases as the auricle distends and also that the recorded initial pressure, i.e., the pressure obtaining at the onset of auricular systole, is practically constant. Initial length of fiber was apparently the only variable at work influencing the strength of muscular contraction. Unfortunately I had overlooked the factor of increasing intra-auricular surface accompanying the increase in initial length of auricular fiber. We know that whereas the circumference of a sphere increases directly as the radius, the surface of a sphere increases as the square of the radius. Applying this fact to observations on the auricle as illustrated in figure 3 in which initial intra-auricular pressure remains constant, it is obvious that initial length of fiber is not the only variable. On the contrary, since the same amount of muscle is spreading over an increasing surface all of which sustains a constant pressure, the initial

tension of the individual fibers increases as the square of the radius whereas the length of fiber increases only as the first power of the radius. Although Patterson, Piper and Starling (9) try to differentiate between the effects of initial tension and initial length of fiber, it appears that they, too, had overlooked the effect which an increasing surface must have upon the initial tension of the individual fibers.

We might in various ways argue the relative effects of initial tension and of initial length of muscular fiber, yet it is more satisfactory to dis-

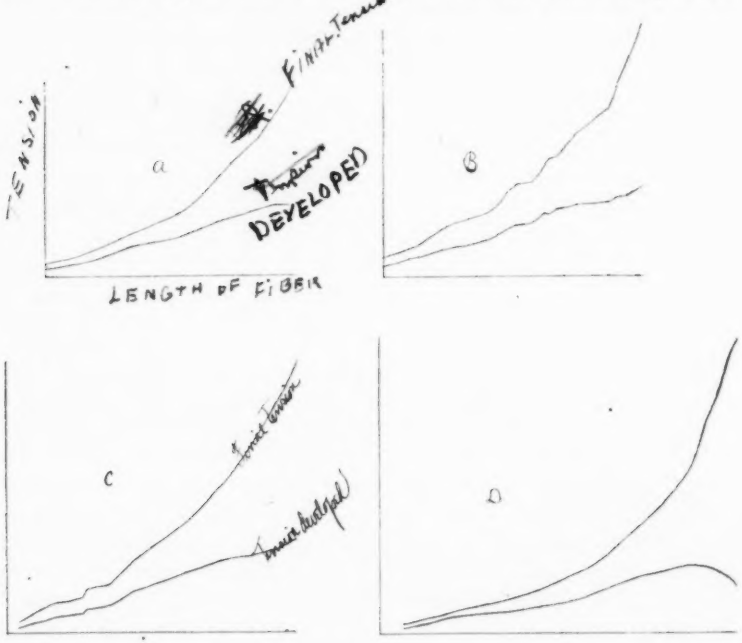

Fig. 1

sociate these factors by direct experiment. We have noted in some few experiments that as the length of fiber increased, a synchronous decrease in initial intra-auricular pressure occurred. Such results were obtained when the auricle was undergoing oscillations of tone by allowing saline to enter the auricle during the phase of tonic relaxation. Since the intra-auricular pressure may decrease out of proportion to the increasing surface and since tonic relaxation per se is detrimental

rather than beneficial to strength of contraction, it appears that the increasing strength of contraction invariably accompanying increasing length of fiber is primarily due to the increasing initial length of fiber.

Having dissociated the effects of initial tension and initial length of fiber under the conditions just described it is of interest to analyze the quantitative data on the relation of strength of contraction to initial length of fiber.

The results of a series of contractions with progressively increasing length of muscle fiber are tabulated and plotted in table 1 and figure 1, *a*. At contraction number 3, the auricular volume is 480 cmm. and

TABLE 1

(1) CONTRACTION	(2) INITIAL INTRA-AURICULAR PRESSURE	(3) FINAL INTRA-AURICULAR PRESSURE	(4) DIFFERENCE BETWEEN INITIAL AND FINAL AURICULAR PRESSURE	(5) AURICULAR VOLUME	(6) INTRA-AURICULAR STRIPACE	(7) LENGTH OF AURICULAR MUSCLE	(8) INITIAL TENSION SUSTAINED	(9) FINAL TENSION SUSTAINED	(10) TENSION DEVELOPED
	mm. H_2O	*mm. H_2O*	*mm. H_2O*	*cmm.*	*sq. mm.*	*mm.*	*cmm. H_2O*	*cmm. H_2O*	*cmm. H_2O*
3	1	2.5	1.5	480	296	30.5	296	740	444
6	1.4	3.8	2.4	835	426	36.6	597	1622	1024
9	1.7	5.0	3.3	1180	508	39.9	863	2540	1676
13	2.4	6.0	3.6	1644	672	46.9	1615	4038	2419
19	3.1	7.1	4.0	2200	818.0	50.6	2535	5807	3272
25	3.7	7.7	4.0	2596	914.0	53.6	3382	1038	3656
31	3.9	8.2	4.3	2931	990.0	55.7	3861	8167	4257
37	5.0	9.0	4.0	3161	1042.0	57.2	5210	9378	4168
43	5.6	9.5	3.9	3335	1078.0	58.2	6036	1024	4204
53	6.2	9.9	3.6	3508	1117.0	59.1	6961	10948	3987
63	6.5	10.2	3.7	3623	1140.0	59.8	7410	11628	4218
70	6.7	10.3	3.6	3662	1148.0	60.0	7691	11824	4132

at contraction number 70 the volume is 3662 cmm. Assuming the auricle to be spherical the length of fiber with number 70 is 60 mm. approximately 196 per cent greater than with number 3. The final tension sustained at number 70 is 1627 per cent greater than the tension sustained at number 3. The tension developed is 930 per cent greater than that developed at number 3.

The curves of final tension and tension developed are plotted in figure 1, *a*. Final tension is represented by a curve with the concavity upwards whereas tension developed is represented by an approximately straight line. Final tension represents two processes, sustenance of

initial tension and development of tension. Sustenance of tension presumably is a non-energy consuming process. According to Hill, tension developed represents the energy consuming processes of muscular contraction in which we are particularly interested.

Three similar experiments are represented in figure 1, *b*, *c* and *d*. The tables are omitted. The fluctuations in curves 1, *b* and 1, *c* are due to tonus oscillations and do not concern us here. Ignoring then these oscillations we note again that tension developed is represented by a straight line. Though in some few experiments (see fig. 4) the curve of tension developed is not as straight as in figure 1, *a*, *b*, *c*, *d*, and though the computations for the curves are based on the assumption that the auricle is perfectly spherical, the results on the whole indicate that cardiac muscle behaves to extension as does striated muscle; that in cardiac muscle tension developed is a linear function of initial length of muscle fiber. Of particular interest as concerns the theories of muscular contraction is the fact that this relation holds for such great variations in initial length of muscle fiber, far greater than those occurring in striated muscle.

THE RELATION OF INITIAL TENSION TO STRENGTH OF MUSCULAR CONTRACTION

When an auricle executes a series of isometric contractions with a constant volume the strength of contraction remains constant. If, during such a series of contractions, a tonus oscillation occurs, the strength of contraction varies even though the length of muscle fiber must necessarily remain unchanged. It is found that final tension and tension developed increase with the rise of initial tension synchronous with tonic contraction. These results suggest the beneficial effect of initial tension upon muscular contraction but unfortunately the change in initial tension is a result of a tonus oscillation which in itself may be the factor influencing the clonic contraction.

Another method may be used for dissociating the effects of initial tension and initial length of fiber. If a series of isometric contractions is obtained first with increasing and then with decreasing length of fiber, as in figure 2, it is seen that for any given initial length of fiber the initial tension is higher in the first series of contractions in which the auricular volume is increasing, than in the second series with decreasing volume. Other things being equal, it would follow that if initial tension exerts a beneficial effect on muscular contraction the

Fig. 2

tension developed per given length of muscle fiber should be greater in the series with increasing length of fiber than in the series with decreasing length of fiber. Before discussing the results, attention should be called to the method employed in obtaining such records and its influence on the interpretation of the results. In the first series with increasing length of fiber, the reservoir is raised to insure an adequate filling pressure. The valve is turned to the maximum position permitting the solution to enter but not leave the auricle. Aside from the diminution in volume permitted by capacity change of the manometer, the contractions are isometric. In the second series with the initial length diminishing, the reservoir is lowered to zero pressure and the valve turned to mean position allowing a small but continuous leakage

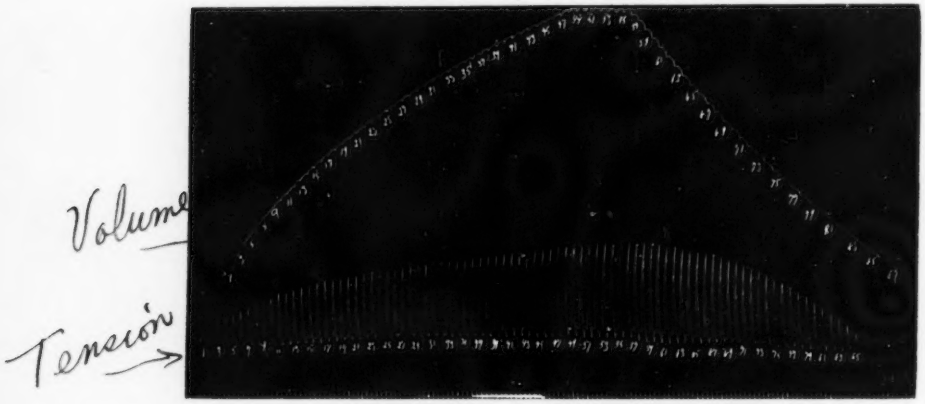

Fig. 3

from the heart back to the reservoir. The resistance in the valve is high enough to permit the development of large pressure changes during contraction so that the contractions approach isometricity but not as closely as the contractions associated with increasing length of fiber. In consequence, a recorded tension in the second series indicates a stronger contraction than the same recorded tension in the first series.

The data obtained from figure 2 are given in table 2 and plotted in figure 4, *a*, *b* and *c*. Figure 4, *a* represents the curves of final tension, 4, *b*, curves of initial tension and 4, *c*, curves of tension developed. The curves of the first and second series are designated *1* and *2* respectively. Final tensions sustained in the two series are nearly equal. The curve

of initial tension sustained is, however, markedly lower in series 2 than in series 1. The curve of tension developed therefore is greater in series 2 than in series 1. Such results might suggest either that initial tension may have a detrimental effect upon muscular contraction or that since the curves of final tension sustained run nearly the same course that muscle at a definite length, other things being equal, is able to sustain a definite final tension—the actual tension developed being dependent upon the level to which the initial tension falls between contractions.

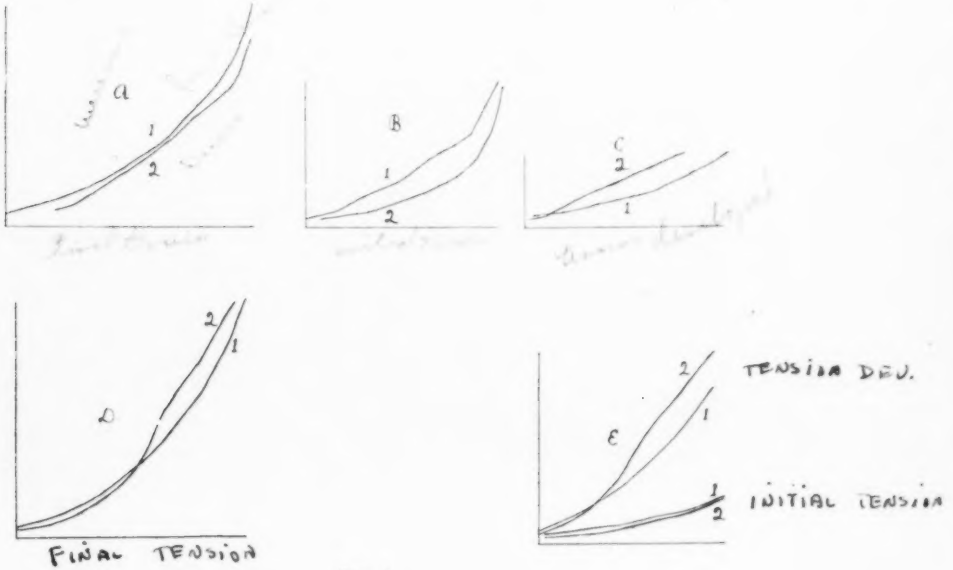

Fig. 4

The experiment represented in figures 3 and 4, *d* and *e*, and table 3 bear on these two suggestions. Here again initial tension is higher in the first series than in the second series. The difference in initial tensions, however, is so slight that a marked effect upon the strength of contraction could hardly be expected. The greater tension developed in the second series is primarily due, in this experiment, to the higher final tension rather than the lower initial tension.

Not knowing of the work of du Bois Reymond (10) and Osborne and Sutherland (11), I sought for more data in the behavior of inflated and

TABLE 2

(1) CONTRACTION	(2) INITIAL INTRA-AURICULAR PRESSURE	(3) FINAL INTRA-AURICULAR PRESSURE	(4) DIFFERENCE BETWEEN INITIAL AND FINAL AURICULAR PRESSURE	(5) AURICULAR VOLUME	(6) INTRA-AURICULAR SURFACE	(7) LENGTH OF AURICULAR MUSCLE	(8) INITIAL TENSION SUSTAINED	(9) FINAL TENSION SUSTAINED	(10) TENSION DEVELOPED
	mm. Hg	*mm. Hg*	*mm. Hg*	*cmm.*	*sq. mm.*	*mm.*	*cmm. H_2O*	*cmm. H_2O*	*cmm. H_2O*
1	3.5	4.9	1.4	230	180	23.9	630	882	252
3	3.8	6.4	2.6	508	308	31.1	1170	1986	815
5	4.1	7.0	2.7	728	395	35.2	1606	2765	1058
8	4.6	7.8	3.2	1062	502	39.7	2324	3900	1576
11	5.0	8.5	3.5	1354	592	43.2	2960	5014	2101
14	5.7	9.0	3.2	1584	657	45.4	3777	5913	2135
17	6.5	9.8	3.3	1800	715	47.4	4647	7021	2373
21	6.7	10.5	3.8	2046	779	49.5	5203	8179	2975
24	6.8	10.8	4.0	2220	823	50.8	5579	8888	3308
27	7.0	11.1	4.1	2392	864	52.1	6048	9590	3542
31	7.1	11.6	4.5	2508	893	53.0	6367	10222	3955
35	8.4	12.8	4.4	2815	965	55.0	8106	12352	4246
39	8.6	13.2	4.5	2938	992	55.8	8560	13074	4513
44	9.5	14.2	4.7	3015	1009	56.3	9585	14378	4792
49	9.7	14.7	5.0	3084	1025	56.8	9942	15067	5025
69	9.3	14.7	5.4	3123	1033	56.9	9576	15154	5578
70	7.7	12.3	4.6	3035	1014	56.4	7788	12472	4684
72	7.2	11.7	4.4	2920	988	55.7	7155	11559	4406
73	6.5	10.8	4.3	2838	970	55.2	6305	10446	4141
74	5.0	10.0	5.0	2623	919	53.8	4595	9190	4595
75	4.5	10.0	5.5	2461	881	52.6	3965	8810	4845
76	4.0	9.8	5.8	2296	840	51.4	3360	8232	4872
77	3.7	9.6	5.8	2123	798	50.1	2992	7644	4652
78	3.7	9.5	5.8	2008	770	49.2	2811	7315	4504
80	3.2	8.8	5.6	1750	702	47.0	2267	6177	3910
82	2.9	8.4	5.5	1508	636	44.7	1884	5342	3458
84	2.6	7.8	5.2	1285	572	42.4	1484	4446	2962
86	2.2	7.2	5.0	1108	518	40.4	1129	3729	2600
88	2.0	6.5	4.5	920	457	37.9	958	3004	2066
90	2.0	5.6	3.6	750	399	35.4	798	2254	1456
92	1.8	4.3	2.5	608	347	33.1	625	1492	876
94	1.8	3.8	2.0	480	296	30.5	524	1124	600
96	1.6	2.4	0.8	393	259	28.6	415	642	227

deflated balloons. If one inflates and then deflates a balloon and plots the pressures on the ordinates against the radius or circumference on the abscissas, curves similar to those in figure 5 are obtained. In that the pressures on deflation are lower per given circumference than on inflation they are analogous to the initial pressures in the auricle with increasing and decreasing length of fiber. In this connection it is interesting to note that Langelaan (12) noted a similar hysteresis in the

TABLE 3

(1) CONTRACTION	(2) INITIAL INTRA-AURICULAR PRESSURE	(3) FINAL INTRA-AURICULAR PRESSURE	(4) DIFFERENCE BETWEEN INITIAL AND FINAL AURICULAR PRESSURE	(5) AURICULAR VOLUME	(6) INTRA-AURICULAR SURFACE	(7) LENGTH OF AURICULAR MUSCLE	(8) INITIAL TENSION SUSTAINED	(9) FINAL TENSION SUSTAINED	(10) TENSION DEVELOPED
	mm. Hg	*mm. Hg*	*mm. Hg*	*cmm.*	*sq. mm.*	*mm.*	*cmm. H_2O*	*cmm. H_2O*	*cmm. H_2O*
6	0.9	2.5	1.6	640	359	33.6	323	897	574
10	1.0	3.3	2.3	980	477	38.7	477	1559	1081
15	1.0	4.1	3.1	1372	596	43.3	596	2443	1847
20	1.1	4.7	3.6	1712	692	46.6	747	3252	2505
25	1.2	5.3	4.1	2028	775	49.3	930	4107	3177
30	1.2	5.7	4.5	2320	847	51.6	1050	4827	3776
35	1.3	6.2	4.9	2588	912	53.5	1185	5700	4524
40	1.4	6.6	5.2	2852	972	55.3	1360	6444	5083
45	1.5	7.2	5.7	3062	1022	56.7	1533	7358	5825
50	1.5	7.6	6.1	3120	1055	57.7	1582	8018	6435
55	1.5	8.0	6.5	3220	1055	57.7	1624	8440	6815
58	1.4	7.8	6.4	3020	1011	56.3	1415	7906	6490
60	1.3	7.7	6.4	2820	966	55.1	1255	7486	6230
65	1.1	7.0	5.9	2260	833	51.1	916	5831	4914
70	1.0	6.2	5.2	1740	699	46.9	699	4333	3634
75	0.8	4.6	3.7	1460	623	44.2	529	2865	2336
80	0.7	2.0	2.2	780	409	35.9	306	1227	920
82	0.7	2.8	2.0	592	340	32.7	248	952	697
85	0.7	1.3	0.6	340	235	27.2	164	295	141

striated muscle of the frog. The question naturally arises, is this hysteresis common to muscle and rubber of significance in the processes of muscular contraction? There is reason to believe (13) that in the case of rubber the change in structure upon which the difference of "initial tension" depends is only temporary (curve *1* can be duplicated after curve *2*) and we might be justified in inferring that in the series of auricular contractions with decreasing length of fiber the structure

of the muscular elements upon which the contractile processes depend is likewise different from the structure of the muscle during the decrease in length of fiber.

There is another possible explanation also based upon the previous history of the muscle, in this case chemical rather than physical. The staircase phenomenon, for example, is explained on the basis of liberated metabolites formed during contraction, which collect in the muscle and influence subsequent contractions. Applying this to the auricle—as the muscle contracts with progressively increasing length of fiber the amount of metabolites liberated with each contraction presumably increases. In a series with decreasing length of fiber the reverse would occur. But the difference between the two series may be that in series 1 with increasing length of fiber the muscle, so to speak, never catches up with itself in the supply of these products, for with increasing length of fiber, the demands on the muscle are steadily increasing while in the series with decreasing length of fiber it is always ahead of itself for then the demands on the muscle are steadily diminishing.

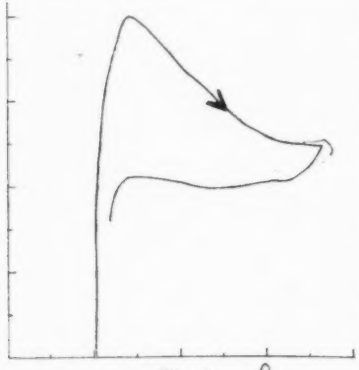

Fig. 5

These explanations of the experiments bearing upon initial tension are admittedly speculative. The results of the experiments, however, have a definite value in pointing to a possible detrimental effect of initial tension upon muscular contraction rather than a beneficial effect. The results enhance the value of the quantitative experiments on the effect of initial length of fiber upon strength of muscular contraction.

A NOTE ON TONUS

A note on tonus. There is an apparent analogy in the behavior of rubber, auricular muscle and the peculiar catch muscle of the bivalve mollusk Pecten described by Parnas (15), Uexkuell (16) and others which may be of significance. The muscle of the Pecten though powerful in that it can support a heavy weight is relatively weak in that its lifting power is small. Recalling the curves of inflation and

deflation of the rubber balloon, the analogy is apparent. Since on deflation the balloon contracts and expels air and is therefore lifting, rubber at a given length can sustain a heavier weight than it can lift. How real this analogy is we of course do not know, yet it is very interesting to perform the Pecten experiment upon the balloon in the following manner: If the partially inflated balloon be enveloped and supported by the hand and then compressed to a slightly smaller volume by forcing water into the manometer with which it is connected (which is comparable to passively closing the shells of the Pecten), when the hands are released the water fails by several centimeters to fall to the original level. The rubber again holds more than it can lift.

To be sure the catch mechanism is developed more highly in the catch muscle of the Pecten yet this does not necessarily spoil the analogy for the mechanism is developed to different degrees in rubber just as it is in muscle under varying conditions. From the description of Parnas (15) it appears that in the weakened Pecten an appreciable interval of time must elapse after passive closing of the valves before the muscle will resist stretching. In that respect the analogy between rubber and muscle may be extended, for if an inflated balloon is deflated and promptly reinflated, the curve of reinflation is little higher than the curve of deflation. But if, as Osborne and Sutherland (11) showed, rubber is allowed to "rest" the first curve of inflation may be duplicated. Apparently opportunity must be given for the molecules to rearrange themselves or approximate each other more closely if the force of attraction is to increase. If a balloon just deflated be subjected to a mechanical shock such as slapping sharply in the hand, the first curve of inflation may be promptly approximated. Strong light also tends to restore the properties of recently stretched rubber. It may be that these differences in the properties of rubber under different conditions are due to the formation of molecular aggregates for it has been found (17) that shaking a rubber solution may double its viscosity through the formation of these aggregates. It has also been noted that rubbing of stretched rubber produces microscopic structural changes (13). It may be that the catch mechanism of muscle in its ultimate analysis is not comparable to the phenomenon shown by rubber, yet attention should be called to a possible analogy between a purely chemical or physical phenomenon and a so-called physiological phenomenon.

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THE RÔLE OF THE PANCREAS IN HYPERGLYCEMIA FROM ETHER

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The part played by the pancreas in producing hyperglycemia and glycosuria in diabetics has been the subject of much study. But there has been a tendency to forget the pancreas when dealing with glycosurias and hyperglycemias not directly associated with diabetes (1). We, as well as others, have been guilty of this. In too many instances the attention has been centered only on liver glycogen, nerve supply to the liver, and adrenal secretion. It is well known that the removal of the pancreas will always cause a severe and uncontrollable hyperglycemia and glycosuria. This leaves no doubt that the pancreas exerts an ever-present influence on the mobilization of dextrose in the body. There is no reason to consider that the pancreatic influence may not be subject to the same variation as the influence of any other organs or tissues of the body. Therefore, we have been led to investigate the relation of pancreatic influence on the hyperglycemia resulting from ether anesthesia.

EXPERIMENTAL WORK

A number of healthy dogs were depancreatized. The removal of the organ was accomplished in two operations. At the first, the entire pancreas was removed except the tip of the tail of the gland. This, with its blood supply still intact, was drawn through the abdominal wall and sutured under the skin. At the second operation, about a week later, the remaining portion of the pancreas was removed and the pedicle ligated.

On account of the mortality following anesthesias, we were forced to make observations after the first operation, or on partially pancreatectomized dogs, on one group of animals and observations on completely pancreatectomized dogs of another group.

The group of partially pancreatectomized animals were not glycosuric. They appeared to be in a healthy condition. The effect of ether anesthesia on the blood sugar was determined. About 10 cc. of blood were taken through a hypodermic needle and oxalated. Ether then was administered by inserting the head of the animal into a cylinder into which air that had passed through ether was forced. After

TABLE 1

Effect of ether on glycemia of partially pancreatectomized animals

ANIMAL	DEXTROSE IN BLOOD		
	Before ether	After 15 minutes ether	Increase
	per cent	*per cent*	*per cent*
1	0.110	0.189	0.079
2	0.100	0.120	0.020
3	0.083	0.111	0.028
4	0.081	0.112	0.031
Average	0.0935	0.1330	0.0395

TABLE 2

Effect of ether on glycemia of completely pancreatectomized animals

ANIMAL	DEXTROSE IN BLOOD		
	Before ether	After 15 minutes ether	Increase
	per cent	*per cent*	*per cent*
5	0.344	0.400	0.056
6	0.344	0.392	0.048
7	0.256	0.322	0.066
8	0.285	0.327	0.042
9	0.287	0.300	0.013
10	0.377	0.400	0.023
Average	0.3155	0.3568	0.0413

fifteen minutes of surgical anesthesia, a second sample of blood was taken in the same manner as before. The dextrose content of the blood was determined by Benedict's method (2). The results expressed in percentage of dextrose in blood are given in table 1.

The second group of animals consisted of those which had had a complete pancreatectomy. They all had glycosuria. They were all active, bright and ready to eat and drink. They were given a meat diet exclu-

sively. Two of the animals were treated exactly like those of the preceding group. On others of this group it was thought best to measure the changes in the output of urinary sugar as well as blood sugar. Therefore a catheter was passed through the urethra into the bladder and left in place throughout the test. The bladder was first emptied and was then washed out with 10 cc. of water three times. The ani-

TABLE 3

Dextrose changes in blood and urine of pancreatectomized animals induced by ether

ANIMAL	DEXTROSE IN BLOOD		DEXTROSE IN URINE OF ½ HOUR BEFORE ETHER	DEXTROSE IN URINE OF ½ HOUR DURING ETHER
	Before ether	After ½ hour ether		
	per cent	*per cent*	*grams*	*grams*
7	0.256	0.327	0.411	0.062
8	0.285	0.340	0.719	0.242
9	0.287	0.303	0.336	0.227
10	0.377	0.410	1.032	0.515

TABLE 4

Dextrose changes in blood and urine of pancreatectomized animals induced by ether

ANIMAL	INCREASE IN DEXTROSE IN BLOOD DUE TO ½ HOUR ETHER	DECREASE IN DEXTROSE IN URINE DUE TO ½ HOUR ETHER	CHANGE IN BODY DEXTROSE DUE TO ½ HOUR ETHER
	grams	*grams*	
7	0.574	0.349	+0.225
8	0.363	0.477	−0.114
9	0.101	0.109	−0.008
10	0.267	0.517	−0.250
First average.....	0.3262	0.3630	−0.0368
Second average.....	0.2436	0.3676	−0.1240

NOTE: The first average is the average of all. Second average omits the values for the first animal.

mal was kept on the board half an hour and at the end of that time the urine was drawn off and the bladder washed out exactly as before. The urine and bladder washings were combined and analyzed for dextrose according to Benedict's method (3). A sample of blood was taken and ether was given in the usual manner described above. After fifteen minutes of anesthesia a second sample of blood was taken. At the end of half an hour of anesthesia a third blood sample was taken and the urine collected as before. The results expressed in percentage

of dextrose in blood are given in table 2. The results expressed in percentage of dextrose in blood and grams of dextrose in the urines for two half-hours are given in table 3. Deductions from table 3 are given in table 4.

DISCUSSION

In table 1 is expressed the effect of fifteen minutes of ether anesthesia on the blood sugar of animals possessing only a small fraction of pancreas and that in an abnormal location. The animals were undieted. There was the usual individual variation which makes individual changes of much less significance than the average from a group of animals. However, it is worth noting that there was a decided increase in the glycemia of every animal. The average blood sugar before anesthesia was 0.0935 per cent and after anesthesia 0.133 per cent of blood dextrose. The average increase in these animals was 0.0395 per cent. In a previous paper (4) the effect of fifteen minutes of ether anesthesia on the blood sugar of seventeen normal dogs was reported. The average blood content of dextrose before anesthesia was 0.090 per cent and after fifteen minutes of ether anesthesia produced in our usual way the bloods averaged 0.127 per cent. The increase was 0.037 per cent. The close agreement between the effect of ether on normal dogs and dogs with much less than the normal pancreas, leads us to conclude that an influence on carbohydrate metabolism equal to normal was being exerted by these small pieces of pancreas implanted under the skin. Ether disturbed the equilibrium just the same as in normal dogs.

In table 2 is expressed the percentage of blood sugar before and after anesthesia of animals without a pancreas. The high glycemia is striking. It is fully three times the amount normally found. All the animals had severe glycosuria. The average dextrose content of the blood before ether was 0.3155 per cent and after fifteen minutes of ether anesthesia 0.3568 per cent. There was an average increase of 0.0413 per cent. It is notable that the values before and after ether were particularly high and that the increase agrees well with the hyperglycemia of normal animals. If there were no changes in the rate of dextrose excretion by way of the urine, this agreement between the increase of normal and pancreatectomized dogs would be of considerable significance.

In order to determine whether there was any change in the dextrose excretion brought about in these pancreatectomized dogs by ether anes-

thesia, the dextrose excreted the half-hour before ether and the half-hour during ether anesthesia was determined. From table 3 it is noted that in the half-hour there was a decided increase in the blood sugar percentages and also a decided decrease in the grams of urinary dextrose. In table 4 it is possible to compare the blood increases with the urine decreases. The blood increases expressed in grams were calculated from the percentages of dextrose, the weights of the dogs and Howell's blood and body weight factor (5) of 7.7 per cent.

With the exception of dog 7, there was in every case less increase in blood sugar than there was decrease of urine sugar. The possible cause of this exception was that during the anesthetic dog 7 stopped breathing and nearly died. The asphyxia in this case was severe and capable of causing marked disturbances of the carbohydrate regulatory mechanism. The average which includes all the animals, shows a decrease in dextrose mobilization of 0.0368 gram due to ether anesthesia of the half-hour. The second average which does not include dog 7 shows a reduction in dextrose mobilization of 0.124 gram. There is no doubt that the removal of the pancreas removed at least one of the sites of action of ether in its production of hyperglycemia.

Cannon (6) worked out the mechanism of the mobilization of dextrose by pain, fear and rage. These factors are capable of stimulating the nerve endings in the adrenals, a stimulation which results in the freeing of epinephrin into the blood stream. This epinephrin acts directly on the glycogen of the liver to set free dextrose. Stewart objects to some of these findings (7).

Macleod (8) has presented data which leads him to conclude that sympathetic nerve terminations controlling glycogenic function can be stimulated either by excess of adrenalin in the blood or by nerve impulses alone.

It is a well-known fact that the removal of the pancreas causes a disappearance of glycogen from the liver and the production of an uncontrollable hyperglycemia and glycosuria. Allen (9) concludes that derangement in the internal secretion from the pancreas is the factor most concerned in the etiology of diabetes. The pancreatic internal secretion must have an inhibitory effect on the liberation of glycogen, since the removal leads to a rapid disappearance of glycogen.

Thus we have strong experimental evidence that there are three important influences being exerted on stored glycogen, i.e., adrenalin, sympathetic nerve endings and pancreatic internal secretion. The nerve endings and adrenalin exert an influence to liberate dextrose from gly-

cogen. Pancreatic internal secretion exerts an influence to prevent the liberation of dextrose from glycogen. So there is for glycolysis an inhibitory and an acceleratory mechanism, a mechanism which is common to many of the body functions. If this be true, no discussion or investigation of any condition which has to do with the mobilization of dextrose is complete unless all these factors are considered. There is no more reason for regarding the internal secretion of the pancreas as invariable than for so regarding the internal secretion of the thyroid.

Our results show that ether does not bring about the mobilization of sugar in the animal without a pancreas, as it does in a normal one. Since the pancreas inhibits glycolysis, we conclude that ether reduces the activity of the pancreatic internal secretion of normal dogs, and thus increases dextrose mobilization. It may be objected that the store of glycogen was so depleted after pancreatectomy that dextrose could not be liberated in normal amounts. This objection will not hold because large amounts of dextrose were being freed into the blood and excreted by the urine hourly as was indicated by the blood and urine content stated in the tables.

Keeton and Ross (1) presented data suggesting that the action of ether to cause hyperglycemia was through hepatic nerve influence and not chemical action directly on the liver cells. They did not take into consideration the internal secretion of the pancreas. Because they did not get hyperglycemia with ether in bi-splanchnectomized animals, they concluded that ether could not be working on the liver directly. The most probable cause for this result, under these conditions, was that the cutting of the nerves to the adrenals reduced the epinephrin output more than ether reduced the internal secretion of the pancreas. Since the adrenals encourage glycolysis and the pancreas inhibits it, the result would be an absence of a hyperglycemia, a result which was obtained.

In a previous publication (10) it was found that chloroform hyperglycemia was not reduced by atropin as was the case with ether hyperglycemia. The mechanism of the two must have certain differences. In view of the present conclusions and since we know chloroform seriously injures liver cells, it is possible to say that the results were due to the inability of the injured nerve endings in the liver and the adrenalin to accomplish as much change in glycogen stored in cells whose walls had been injured and whose chemical equilibrium had been altered, as normally. So that possibly chloroform reduces the pancreatic internal secretion exactly the same as ether but in addition injures the liver,

which would result in not so great a hyperglycemia as that of ether and also not be subject to influences of such drugs as atropin while ether is so influenced.

Stewart and Rogoff (7) have presented much data contending that ether hyperglycemia is not due to the liberation of excess adrenalin into the blood stream. They have cut the splanchnics on one side and removed the adrenal gland on the other side. The cats were allowed a number of days to recover. Then they were anesthetized with ether. A hyperglycemia resulted. The amount of adrenalin in the blood before and after the anesthesia was far below normal. During the time allowed for recovery there is no doubt that there was a readjustment between the accelerator and inhibitor glycolytic mechanisms, as is commonly seen after a disturbance of one of the mechanisms governing heart rate. So at the end of several days the inhibitor glycolytic mechanism, the internal secretion of the pancreas, had fallen to a lower level more nearly equal to the accelerator glycolytic influence of adrenal and hepatic nerve ending activity. So when ether was given, according to our view the activity of the pancreas was reduced and the accelerator glycolytic mechanism became the more powerful and dextrose was set free into the blood stream in greater abundance.

SUMMARY AND CONCLUSIONS

The hyperglycemia induced by ether was measured on two series of dogs. One group had partial pancreatectomies and the other group had complete pancreatectomies. The effect on the urine dextrose of ether anesthesia was also measured in the case of the pancreatectomized dogs.

The hyperglycemia induced by ether anesthesia of partially pancreatectomized dogs was the same as that of normal dogs.

The hyperglycemia induced by ether anesthesia of completely pancreatectomized dogs was practically the same as normal. Comparing the dextrose output in the urine half an hour before anesthesia and half an hour during anesthesia showed a marked decrease in the elimination of dextrose. Comparing the rate mobilization of dextrose with and without ether showed that it was markedly decreased by the drug.

The results lead us to conclude that the chief action of ether in causing a hyperglycemia in normal animals is to reduce the influence of the internal secretion of the pancreas on glycolysis.

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PHYSIOLOGICAL STUDIES ON PLANARIA

IV. A Further Study of Oxygen Consumption during Starvation

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INTRODUCTION

In the first paper of this series (1) it was shown that in Planaria dorotocephala the rate of oxygen consumption rises during starvation. This rise is readily detectable in less than three weeks after the cessation of feeding; and from this time on the oxygen consumption rises continuously with an acceleration for at least eight weeks, when the experiments were concluded. This result agrees with the result previously obtained by the susceptibility method. The susceptibility method consists in observing the time of death of organisms in lethal solutions. It is believed on adequate grounds, which need not be reiterated here since they have been discussed in numerous papers from this laboratory, that such time of death is in a general way a measure or indication of the metabolic rate of organisms. By this method it had been found many years ago by Professor Child that the susceptibility of planarians is increased when they are starved and that it is greater and their survival time in lethal solutions shorter the longer the period of starvation has endured. From this result Child drew the conclusion that the rate of metabolism is increased by starvation. My results on the rate of oxygen consumption confirmed the conclusion reached by Child through the susceptibility method and further supported Child's general conceptions concerning the nature of senescence and rejuvenescence (3).

Meantime, however, Lund and Allen at the University of Minnesota sought to find evidence against Child's viewpoint. Their point of attack concerns the susceptibility method and they have been attempting to show that the susceptibility method is not a reliable measure of metabolic rate. Their arguments reveal a certain amount of misunderstanding concerning the application of the susceptibility method but

the matter is perhaps of little consequence now, since the main conclusions drawn by the use of the susceptibility method have been verified by quantitative determinations of oxygen consumption and carbon-dioxide production.

In a paper appearing simultaneously with mine already referred to (1), Allen (2) sought to show that in Planaria agilis and Planaria maculata the rate of oxygen consumption is not increased during starvation within the periods tested by him (nine weeks). In this paper he also made an elaborate comparison between the susceptibility of planarians in various physiological conditions and their rate of oxygen consumption under the same conditions and argued that since the two methods do not (according to him) always yield the same results, the susceptibility method is not a reliable index of the rate of respiratory metabolism.

This comparison made by Allen would probably appear convincing to anyone not conversant with all of the facts. But it has in reality no adequate scientific basis since the comparison is made *between the susceptibility data obtained by us on Planaria dorotocephala and the oxygen consumption data obtained by him on Planaria agilis and Planaria maculata.*[1] Allen at the time of writing did not know anything about the *rate of oxygen consumption* of P. dorotocephala during starvation. How then can he maintain that it does not agree with the findings by the susceptibility method? As a matter of fact there is in this species as I have shown (1) general agreement between the susceptibility results and the rate of oxygen consumption; both show that metabbolism is markedly increased by starvation. On the other hand Allen did not know or attempt to find out anything about the *susceptibility* of P. agilis during starvation. How then can he maintain that in this species there is a discrepancy between the results by the susceptibility method and the oxygen consumption data? My own findings demonstrate that no such discrepancy exists. It is true that the rate of oxygen consumption in P. agilis increases very slowly during starvation and the increase appears only after four to six weeks while in P. dorotocephala it is present before the third week of starvation; but it is also true that in P. agilis the *susceptibility to potassium cyanide shows no or little increase during this period.* This fact completely demolishes Allen's argument. If he had taken the trouble to investigate the susceptibility of P. agilis during starvation, he would have realized

[1] Owing to the uncertainty in the identification of the species Planaria maculata, explained later, consideration of this species is left out of the discussion.

that he had no basis for his contentions. As a matter of fact, the conditions in P. agilis, far from disproving the value of the susceptibility method, furnish a remarkable demonstration of its utility; for the differences between the two species could have been discovered by means of the susceptibility method alone.

Marked differences exist in the physiology of P. dorotocephala and P. agilis; these differences are mostly quantitative and not qualitative. P. agilis respires about two-thirds as fast as P. dorotocephala, it consumes more food and has probably larger food reserves, it loses weight more slowly in starvation, and requires a much longer period to reach the same degree of rejuvenescence. Such differences must receive consideration in experimental work. To assume, as Allen did, that because P. dorotocephala reaches a high degree of rejuvenescence within a few weeks, other species must also, and if they do not the conclusions drawn from P. dorotocephala are erroneous, is an unscientific proceeding. Each species must be thoroughly analyzed and studied as we have studied P. dorotocephala before comparisons can be made.

The question next arises: is it true that in P. agilis and P. maculata, as maintained by Allen, the oxygen consumption does not increase within nine weeks of starvation? I have repeated Allen's experiments upon these species (cf., however, footnote 1) and find that he was mistaken in his conclusions. A critical examination of the data presented by him shows that they are inadequate and unconvincing and furnish an insufficient basis for his contentions, especially in view of the fact that those contentions are counter to a large body of evidence already at hand.

Allen has presented six experiments, three on P. agilis and three on P. maculata. In but one of the six has the oxygen consumption been tested from the beginning to the end of the period of starvation. This experiment is given in his table 7. At the end of this experiment the worms were consuming some 40 per cent more oxygen than during the early part of the experiment. The result, therefore, in the one really adequate experiment performed by Allen is exactly the contrary to his general conclusion. My experiments show that the results given in this table are essentially correct.

I wish to consider each of his experiments in a little more detail. Tables 4, 5 and 6 deal with P. maculata. In table 4 the experiment is begun with worms which had already been starving for "several weeks." We are not told just how long this period was nor do we know anything about the rate of oxygen consumption during this part of the starva-

tion period. The oxygen consumption of these worms was then tested during thirty-four days of starvation, during which it is said by Allen to be "constant." This "constancy" means a variation of from 5.8 to 8.2 cc. of oxygen per gram per day. This variation is much greater than that regarded in other tables as of significance and clearly indicates a lack of experience with the method. Leaving this irregularity out of consideration, it may be said that the data in the table are of no significance since after "several weeks" of starvation a high level of metabolism has already been attained by some species of Planaria and this rate may not increase further. Unless we know the complete history of such worms the data are of no value for the purpose. The reader should note, however, that according to table 5 the average oxygen consumption per gram per day after "several weeks" starvation is *6.9 cc.* Turning now to table 5, we find that in the same species of Planaria between the fifteenth and forty-second days of starvation the average oxygen consumption per gram per day is about *4.5 cc.*; and again in table 6, between the fifth and twenty-ninth days of starvation it is *5.2*. This discrepancy between the results in table 4 on the one hand and those in tables 5 and 6 on the other was not mentioned by Allen; but it is perfectly evident that it can be explained only on the basis that the oxygen consumption is decidedly increased after "several weeks" starvation. We may therefore state that Allen's data on P. maculata are inadequate because in no case was the oxygen consumption determined throughout a period of starvation covering a number of weeks; and that as far as they show anything at all they certainly do not support Allen's conclusion but lead in the opposite direction.

Allen's tables 2, 3 and 7 deal with P. agilis. In table 2 it is shown that the oxygen consumption in this species is less after three weeks than after one day of starvation. This is also the case in all species of Planaria which have been tested. The greater rate after one day of starvation is due to the persistence of the effect of feeding on the digestive tract as explained in a previous paper (1). This table has therefore no direct bearing on the question of the rate of metabolism during starvation, since the comparison must be made with worms in which the increased oxygen consumption due to feeding has been eliminated. In Allen's table 3 data are given which show no difference in the rate of oxygen consumption between worms starved one week and worms starved nine weeks. These data are most certainly incorrect. They are directly contradicted by the results in Allen's table 7, in which

after nine weeks' starvation an increase of about 40 per cent in the rate of oxygen consumption has occurred. Perusal of the text referring to table 3 throws some light on the origin of the error, since it is stated that the control worms had been kept in the laboratory only a few days preceding the test. Since the metabolic rate of worms decreases when they are maintained under laboratory conditions it is probable that the control worms in this case had a higher rate of oxygen consumption than they would have had if kept in the laboratory the same length of time as the experimental worms.

As already noted, the experiment recorded in table 7 is the only one presented by Allen in which the metabolism of the worms was studied from the beginning to the end of a starvation period lasting about ten weeks. This experiment is also the most accurate, as Allen states. Unfortunately at the critical point in the experiment, a gap of three weeks is present. Nevertheless the results clearly show that the oxygen consumption of P. agilis is increased by starvation, a rise of about 40 per cent having occurred after nine weeks' starvation. Allen attempts to explain away this result on trivial grounds, but my own experiments on five different lots of P. agilis, each of which was studied throughout a period of at least twelve weeks' starvation, prove that it is essentially correct. The rate of oxygen consumption of P. agilis like that of other species of Planaria is increased by starvation; but a longer period of time is required for the increase to occur.

It is evident that throughout his experiments Allen proceeded on a physiologically incorrect assumption. He supposed that absolute length of time is the important factor in starvation; whereas in fact the primary factor is *the rate at which starvation proceeds.* This rate is determined by a number of factors other than time, such as: the temperature during starvation, the age (size) of the worms at the beginning of the experiment, the metabolic rate of the species in question, the rate at which weight is lost, and the amount of food reserves present in the body. An increase in metabolism as a consequence of starvation cannot be expected to occur until the animal actually begins to use its own tissues for food and the time required for this is variable with different species and under the different circumstances just enumerated.

EXPERIMENTS ON PLANARIA MACULATA

1. Source and care of material. The so-called species Planaria maculata lives in the Chicago region in small still bodies of water. It is found creeping about on the submersed vegetation. The stocks of worms used in these experiments were collected from the lagoon in Jackson Park in the city of Chicago. This lagoon to the east of the Wooded Isle is filled with submersed water weeds, chiefly Elodea, Ceratophyllum, Myriophyllum and Potamogeton. Large quantities of these plants were brought in, packed closely in dish pans, and enough water added to just cover the plants. Within two or three days the plants begin to decay and as the planarians cannot endure such conditions they creep up to the surface layers of plants, and after more time has elapsed will collect at the margins of the pans near the surface. If the surface layers of plants are removed after two or three days, placed in pans with a large quantity of water, and violently agitated, the worms will fall off to the bottom and begin to crawl up the sides of the pan to the top. They can then be readily picked off and placed in pans of clear water. Although the method is rather laborious and time-consuming, it is possible with a little patience to accumulate a considerable stock of such worms. They are most abundant on the vegetation in the autumn.

Stocks so collected were kept in large pans of well water. If they are to be maintained for any length of time, they must be fed. I at first tried to feed them by the same method we were accustomed to use for P. dorotocephala, namely, by placing pieces of liver in the pans. It was at once found that this species will not take food by this method. It was then suggested by Professor Child that this species is so sluggish and insensitive that it does not detect food except at very short distances and that the liver should be ground and strewn over the bottom of the pan. This procedure was tried but was even less successful than the preceding plan, because not only did the worms refuse to feed but many of them died as a consequence of the presence of the ground liver in the pan. While giving this plan a trial, however, the correct method of feeding was accidently discovered. In an attempt to reduce the labor of removing the ground liver from the pans after feeding it was decided to wash the liver, since blood and other materials diffusing from the liver render the water so opaque that the worms can no longer be seen. The liver was then ground and washed and it was at once observed that the worms would feed voraciously on such

washed liver. Evidently some materials in the liver (bile?) are injurious to this species. The feeding plan adopted was then the following: the liver was ground in a meat grinder, poured into a strainer, and a stream of water run through it until the water came through clear. The washed liver fragments were then strewn over the bottom of the pan and the worms stirred up and washed down from their usual resting places along the sides of the pan. After three or four hours, the worms, having fed, again accumulate on the sides of the pan, and the liver fragments can be removed with the aid of a suction pipette made of an atomizer bulb and a glass tube of wide bore. The worms and the pan are then washed thoroughly two or three times to remove all fragments of liver. By this method the worms feed readily and grow with astonishing rapidity.

2. Taxonomy of Planaria maculata. Inspection of a stock of worms collected as described above at once shows that two distinct types are present. Neither of these corresponds to the description of the true Planaria maculata of the eastern United States. I am of the opinion that there are at least three distinct species to which the name Planaria maculata has been applied indiscriminately.

One of the types occurring in collections from pond weeds has the following characteristics: the body is short and relatively broad and not at all or but slightly narrowed behind the auricles; the pigmentation varies from chocolate to ashy brown and is decidedly arranged in spots with conspicuous white blotches between; there is never any white stripe down the center of the dorsal side but large specimens show a tendency to develop a dark stripe in this region. This type is designated in this paper as the spotted variety. It is not found sexually mature in nature but sexually mature individuals have developed in our stocks and are at the present writing laying capsules. An investigation of the morphology of the reproductive system in this form will settle the question of its identity or non-identity with Planaria maculata.

The other species present in our stocks has the following appearance: the body is longer and more slender than in the spotted variety and very much narrowed behind the auricles; the pigmentation is more evenly distributed and to the naked eye not at all spotty in arrangement; it is of a very dark brown, almost black color and the white part of the eyes stands out very conspicuously in consequence; there is always a well-marked white stripe down the center of the back. This form is designated in this paper as the striped variety. Although specimens in our stocks have attained a considerable increase in size, no sexually mature individuals have as yet appeared.

Both of these forms are different from the true Planaria maculata which I have observed at Falmouth, Mass. The general proportions of the latter are similar to those of the spotted variety but it is not nearly so spotty in appearance and generally bears a white stripe down the center of the back. Further, it lives in a different kind of habitat, under stones in clear, comparatively quiet water. This species does not live in the Chicago region as the type of habitat necessary for it does not occur here. Finally its behavior in regeneration experiments is quite different from that of our two varieties.

In view of this confusion regarding the taxonomy of this species, it is impossible to know whether investigators working with what is called P. maculata are really using the same species or not. This difficulty arises in connection with Allen's experiments. It is not certain that the species used by Allen is the same as either of the two varieties used by me in the present experiments. From some remarks made by Allen to Professor Child at the 1919 meeting of the American Society of Zoölogists, it seems probable that he was working with the true Planaria maculata. At any rate the results which I have obtained are quite different from his and this difference may be due in part to the use of different species.

3. Method of procedure. From the general stocks lots of worms were selected, each consisting of some two hundred worms of approximately the same length. Two such lots were selected of the striped variety and three of the spotted variety. Each lot was kept in a separate dish and its rate of oxygen consumption determined at intervals, generally two-week intervals, during a period of starvation. To prevent such worms from undergoing fission, it is only necessary to place them in dishes already well coated with slime from other worms.

The method of determining the oxygen consumption has already been described (1). Briefly the lot of worms was placed in a 500 cc. Erlenmeyer flask, filled air-tight with water of known oxygen content; after an interval a sample was drawn from this flask and its oxygen content determined. The difference between the oxygen content of this sample and the original oxygen content of the water gives the amount of oxygen consumed by the worms. The oxygen content was determined by Winkler's method. Two independent determinations were made each time. After the experiment was completed the worms were weighed. The temperature was the same each time the oxygen consumption of the worms was tested, but between these tests the worms remained at room temperature, which is naturally rather variable.

4. Results. The results of these tests on the oxygen consumption of five separate lots of P. maculata at various intervals during a period of starvation are summarized in table 1. The detailed data will be found at the end of the paper.

5. General conclusions regarding Planaria maculata. As shown in table 1, the oxygen consumption falls after feeding and reaches its lowest level within three or four days. It then remains at this level until after the second week of starvation. From this time on it rises

TABLE 1

Oxygen consumption of the spotted and the striped varieties of Planaria maculata during starvation, showing increase in all cases. All temperatures 20 ± 0.5°C.

TIME SINCE FEEDING	STRIPED VARIETY		SPOTTED VARIETY		
	Lot 1	Lot 10	Lot 3	Lot 2	Lot 4
	10–12 mm. (Original length)	8–12 mm. (Original length)	8 mm. (Original length)	8 mm. (Original length)	10–12 mm. (Original length)
	Oxygen consumed by 0.5 gram in two hours				
	cc.	cc.	cc.	cc.	cc.
1 day	0.18		0.25		
3–4 days	0.16		0.21	0.28	0.21
1 week	0.16		0.21		
2 weeks	0.15	0.22	0.22	0.28	0.21
4 weeks	0.17	0.26	0.24	0.29	0.28
6 weeks	0.22	0.35	0.29	0.36	0.30
8 weeks	0.21		0.29	0.59*	0.42
10 weeks	0.23*		0.52*		0.61*
Per cent increase	53	59	147	110	190

* Means that the worms were decapitated on the day preceding the test so that movement was eliminated.

continuously. A marked difference was found between the two varieties used. In the striped variety the oxygen consumption reached a maximum by the end of the sixth week of starvation and exhibited no further rise within the limits of the experiments. It is therefore not safe to begin these tests on worms which have already starved several weeks, as done by Allen in his experiment 4. In the spotted variety, on the other hand, the oxygen consumption continued to increase with an acceleration throughout the experiments. The experiment was discontinued in each case when the size of the worms was so reduced as

to render further determinations impracticable. In most cases the heads of the worms were removed preceding the last test so that movement is eliminated as a factor in the result. In the early stages of the experiments, some of the stocks were not tested as this was considered unnecessary in view of the fact that the fall in oxygen consumption during this period is so well established that it may be assumed to have occurred. No tests are omitted from the table; all that were made are given.

EXPERIMENTS ON PLANARIA AGILIS

1. Source and care of material. This species is not indigenous to the Chicago region. About three hundred individuals were purchased from the firm of Powers Powers, Lincoln, Nebraska. Since Allen's stock was also obtained from this firm, we are dealing here with the same species. This species is stated by Mr. Powers to live in springs, a habitat similar to that frequented by P. dorotocephala. The two species resemble each other very much in appearance, although agilis is more restless and attains a larger size.

The original individuals were cut into pieces in order to obtain a sufficient number of individuals for the experiments. The experiments were not begun until three or four months after this had been done so that it is impossible that this procedure could have affected the results. The worms were fed by the same method as used for P. maculata and ate voraciously and grew with remarkable rapidity.

2. Digestive tract of Planaria agilis. In order to make a comparison between Planaria agilis and Planaria dorotocephala it is not sufficient, as Allen has done, to perform a few isolated tests. The physiology of the species must be studied before such comparisons are valid. One of the striking differences between these two species concerns the capacity of the digestive tract. Planaria agilis is an extremely voracious feeder, and appears to be very hungry after only three or four days' starvation. It will attack injured worms much more fiercely than will the other species we have observed. After it has fed, the body is noticeably distended so that it is quite easy to distinguish the individuals that have fed from those that have not. This observation suggested that the digestive tract has a larger capacity in P. agilis than in P. dorotocephala. Sections show that such is the case. Figure 1 gives camera lucida outlines of the body wall and digestive tract at various levels of section in individuals of P. agilis and P. dorotocephala of the same length. It is readily seen that the digestive tract of P. agilis, partic-

ularly in regions through and in front of the pharynx, is more capacious than that of P. dorotocephala. In the latter species, the anterior part of the digestive tract has the form of a median trunk with lateral branches, while in the former species there is a large irregular cavity extending nearly the width of the body. By simply distending the whole body, the capacity of this digestive tract would become very great and as already noted such distension occurs when P. agilis feeds.

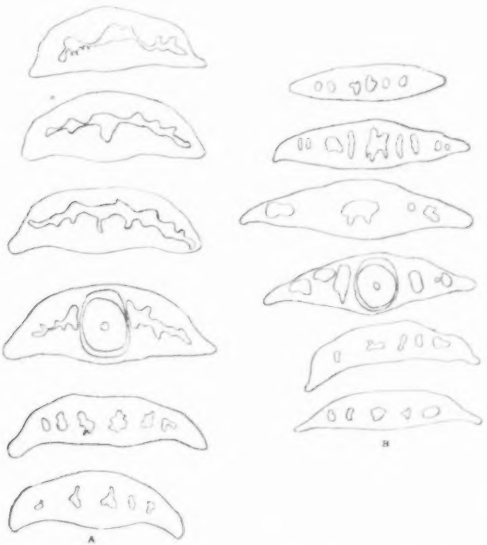

Fig. 1. *A*, six sections through Planaria agilis, drawn with the camera lucida, showing outlines of the cavity of the digestive tract; most anterior section at the top and others in order. *B*, sections through similar levels of Planaria dorotocephala, showing outlines of the cavity of the digestive tract. Both worms 15 mm. long.

Further the body is broader and thicker in P. agilis in proportion to length than in P. dorotocephala. These facts indicate that P. agilis can consume more food at each feeding and accumulates more food reserves than P. dorotocephala; and therefore it must be starved for a longer period before it will reach the same metabolic condition that the other species reaches in a short time.

3. Loss of weight of Planaria agilis as compared with P. dorotocephala. It was noticed that during starvation the reduction in size is much slower in P. agilis than in P. dorotocephala. To obtain a more accurate measure of this difference fifty worms of the same length of each

TABLE 2

Comparison of loss of weight of Planaria dorotocephala and Planaria agilis during starvation. Figures are weights of fifty worms, approximately 15 mm. long at the beginning of the starvation period

TIME SINCE FEEDING	PLANARIA DOROTOCEPHALA		PLANARIA AGILIS	
	Weight	Total decrease	Weight	Total decrease
	gram	*per cent*	*gram*	*per cent*
1 day	0.411		0.550	
1 week	0.322	21	0.503	8
2 weeks	0.284	30	0.472	13
4 weeks	0.214	47	0.380	30
6 weeks	0.159	61	0.289	47
8 weeks	0.107	74	0.227	58
10 weeks	0.073	82	0.169	69

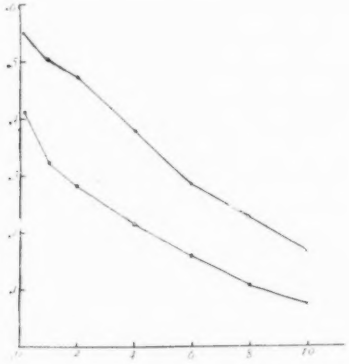

Fig. 2. Graph showing loss of weight of Planaria agilis and Planaria dorotocephala in starvation. Abscissa, time in weeks; ordinate, weight in tenths of a gram. Upper line, P. agilis; lower line, P. dorotocephala; weights for fifty worms.

species were weighed at intervals during starvation. Both lots of worms were kept under the same conditions throughout. The data on the loss of weight are given in table 2 and a graph constructed from the same data presented in figure 2. It is evident that P. doroto-

cephala loses weight in starvation more rapidly than P. agilis and that the difference is greatest in the early part of the starvation period. The loss of weight of P. agilis is practically directly proportional to time so that the resulting graph is a straight line, as Allen has also shown. In P. dorotocephala on the other hand the loss of weight is more rapid at first and proceeds at a uniform rate only later in the starvation period. The slower loss of weight in P. agilis is evidently correlated with its greater breadth and thickness and greater supply of food reserves. Since P. agilis loses weight more slowly than P. dorotocephala, a longer time will be required for it to starve to the same degree and reach the same metabolic condition as the latter species. This was found to be the case.

4. Oxygen consumption of P. agilis during starvation. The methods employed were the same as those described under P. maculata. Five lots of worms, the members of each lot of approximately the same size, were isolated from the general stock and their oxygen consumption determined at various intervals after the cessation of feeding. Fission can readily be prevented in such lots by keeping them in dishes or pans coated with slime. A few fissions will occur and such divided worms were removed. The results of these five experiments are tabulated in table 3. The detailed data are given at the end of the paper.

The results tabulated in table 3 prove that in Planaria agilis as in other species of Planaria the rate of oxygen consumption increases during starvation. A longer period of starvation must elapse, however, in this species before the rise occurs. This length of time required depends, among other factors, on the original size (age) of the worms. Thus in lot 6, where the worms were 10 to 12 mm. long at the beginning of the experiment, the rise is present after four weeks of starvation; in lot 7, with worms 12 to 15 mm. long, the rise begins by the sixth week and in the other lots, with worms 15 mm. long or longer, the rise begins by the eighth week of starvation. In all cases the rise is plainly present by the beginning of the eighth week of starvation. This result is therefore contradictory to the contentions of Allen, who insists that within the length of time tested by him, namely, nine weeks, no such rise is present. I have already pointed out that his contention is directly contradicted by his own data given in his table 7, and that the results in this table are correct.

After the eighth week the rise is at first rather slow or even absent as in experiments 6 and 5 but later the increase is very rapid, and the oxygen consumption finally attained may be more than 100 per cent

greater than the oxygen consumption after a starvation period of one week. After twelve or more weeks of starvation worms of this species are no further reduced in size than are individuals of Planaria dorotocephala of the same original length after six or seven weeks of starvation. The slower rate of reduction of P. agilis explains why the metabolic rate increases more slowly.

TABLE 3

Oxygen consumption of Planaria agilis during starvation, showing increase in all cases by the eighth week

TIME SINCE FEEDING	LOT 6	LOT 7	LOT 5	LOT 8	LOT 9
	18°C.	21°C.	18°C.	21°C.	21°C.
	10–12 mm. (Original length)	12–15 mm. (Original length)	15–18 mm. (Original length)	15–18 mm. (Original length)	18–20 mm. (Original length)
	Oxygen consumed by 0.5 gram in two hours				
	cc.	*cc.*	*cc.*	*cc.*	*cc.*
1 day	0.16	0.24	0.16	0.20	0.23
4 days	0.14		0.14		
1 week	0.14		0.13		
2 weeks	0.12	0.19	0.12	0.17	0.17
4 weeks	0.14	0.18	0.14	0.17	0.16
6 weeks	0.15	0.19	0.12	0.16	0.16
8 weeks	0.17	0.21	0.15	0.18	0.18
10 weeks	0.17	0.23	0.17	0.20	0.19
12 weeks	0.17	0.32	0.17	0.33	0.27
16 weeks	0.31*		0.25*		
Per cent increase.....	158	77	109	106	65

* Means that heads were removed the day preceding the test.

5. Susceptibility of P. agilis during starvation. This matter has already been considered in the introduction. If the susceptibility method is reliable as a means of determining general metabolic rate, the susceptibility of P. agilis should increase more slowly during starvation than is the case with Planaria dorotocephala. I have made some tests of the susceptibility to cyanide of P. agilis during starvation. These tests were not as complete as is desirable but they indicated clearly enough the general situation in this species. All starving worms are more susceptible to cyanide than recently fed ones. Worms starved two weeks to five weeks are slightly more susceptible than

those starved one week. There is, however, *no difference or very little difference in the susceptibility of worms starved two, three, four and five weeks.* Thus between the second and sixth weeks of starvation there is very little increase in susceptibility while during this period in P. dorotocephala a marked increase is demonstrable. Reference to table 3 shows that during this period also the oxygen consumption of P. agilis remains practically stationary. In later periods of starvation the susceptibility of P. agilis increases.

There is thus in this species a general agreement between the results by the susceptibility method and the results from direct measurement of the rate of oxygen consumption.

6. Comparison of starved and young (small)) individuals of P. agilis. It is insisted by Allen in his paper that whereas small worms consume

TABLE 4

Comparison of the rates of oxygen consumption of small (young) recently fed worms, large (old) recently fed worms, and worms reduced by starvation to a size similar to that of the young worms. Temperature 21°C.

KIND OF WORM	LENGTH	TIME SINCE FEEDING	OXYGEN CONSUMED BY 0.5 GRAM PER 2 HOURS
	mm.		
Young	6–10	5 days	0.22
	6–10	5 days	0.21
Old	15–18	2 weeks	0.17
	18–20	2 weeks	0.17
Starved	6	12 weeks	0.33
	6	12 weeks	0.27

more oxygen per unit weight than large ones, worms reduced by starvation consume the same amount of oxygen as before such reduction. This statement appears improbable on the face of it and my experiments show it to be erroneous. Small worms whether their size is due to youth or starvation consume more oxygen per unit weight per unit time than large worms in an adequate state of nutrition. It requires some twelve weeks of starvation to reduce P. agilis from a length of 18 to 20 mm. to a length of 6 mm. Such a reduction involves an increase of at least 60 per cent in the rate of oxygen consumption. According to Allen the difference in oxygen consumption between 6 mm. and 18 mm. worms is about 60 per cent (his table 10) when movement is not eliminated and about 40 per cent (his table 11) when

movement is eliminated. As young fed worms move about much more than greatly starved worms, the latter figures are more correct.

In table 4 are given some data on the oxygen consumption of two lots of young worms 6 to 10 mm. long, starved five days; the oxygen consumption of worms 15 to 20 mm. long, from the same stock, starved two weeks; and the oxygen consumption of the same worms after reduction to a size similar to that of the young worms. After such reduction the worms have a higher rate of oxygen consumption than the young worms of similar size.

DISCUSSION

It is shown in this paper that in two varieties of Planaria maculata (which are probably separate species) and in Planaria agilis the rate of oxygen consumption per unit weight is increased by starvation. This increase begins in the first named forms within four weeks from the cessation of feeding but in Planaria agilis not until the eighth week if the original length exceeded 15 mm. This result agrees with results previously presented on other species (1) and disagrees with the contentions of Allen (2). Planaria agilis differs from all other species which have been adequately tested in that the oxygen consumption remains at nearly the same level between the second and sixth weeks of starvation, and rises only subsequently to this period. This circumstance is due to the fact that Planaria agilis starves more slowly than other species; it has a lower metabolic rate, greater food reserves, and a more capacious digestive tract, and consequently reduces more slowly in starvation than other species of Planaria. The remark of Allen that "the fact that the rate of oxidations is uniform during a long period of starvation will serve as an important basis for the study of respiratory metabolism in these forms" applies to Planaria agilis only. There is no level of "basal" or "standard" metabolism in other species, but their metabolism is continuously changing during starvation. Even in Planaria agilis the rate of oxygen consumption is probably not stationary but is falling very slowly during the first half of this period of apparent constancy and rising very slowly during the latter half of it.

Since Allen's paper is directed mainly against the reliability of the susceptibility method, it remains to make some statements regarding this method. In the first place, the susceptibility method measures the time of death. The time of death can naturally be determined

only for those parts of the organism visible to the observer, namely, the superficial parts. Strictly speaking, therefore, survival time is a *measure of the metabolic rate of the body surface only*. That we have not in earlier publications emphasized this is due simply to the circumstance that the occasion for emphasizing it had not arisen. It had always been recognized by us that the susceptibility method concerns chiefly superficial structures and statements were made in various publications that internal systems and organs may show different susceptibility relations from the body surface.

It is, however, evident that *when special internal conditions are eliminated* survival time is a measure of general physiological and metabolic conditions in the whole organism. In the lower organisms there is only one such special internal condition which it has thus far been necessary to consider—that is the physiological condition of the digestive tract. When this factor is eliminated by keeping it in the same functional state in organisms which are to be compared then, as far as our data at present go, there is never any discrepancy between the results by the susceptibility method and the determinations of total oxygen consumption and carbon dioxide production. A summary of some physiological conditions in which both susceptibility and respiratory rate have been determined is given in table 5.

From table 5 it will be seen that in nearly all cases where the survival time is shorter, the rate of respiratory exchange is greater, and where the survival time is lengthened, the rate of respiratory exchange is decreased, the sole exceptions being those concerned with feeding. That this apparent discrepancy exists was known to us for a considerable time before Lund and Allen published their work. Their viewpoint, that because this discrepancy exists the whole conception is erroneous, is certainly unscientific, to say the least. The correct procedure is to attempt to discover the cause of the discrepancy. This has been done and it has been found that the discrepancy is due to the behavior of the digestive tract. The respiratory rate of the digestive tract is increased by feeding but as the surface of the body is not affected by the feeding process, the susceptibility of the surface does not change. On the other hand the susceptibility of the intestine is increased by feeding, although it is somewhat difficult to observe the digestive tract by the susceptibility method in Planaria. In more transparent forms, like Hydra, the region of the body where food is being digested exhibits a very striking increase in susceptibility. After feeding, the respiratory rate of the digestive tract falls and as the body surface is again

not involved in this process, the susceptibility of the surface is not altered. It is therefore necessary under circumstances involving the digestive tract to consider the body wall and digestive tract separately. As this is continuously done in physiological experiments on higher animals, where in order to study one physiological process it is necessary to have the others constant, we fail to see that such a procedure is "damaging to the theory that susceptibility is a measure of rate of

TABLE 5

Summary of the results from measurements of the survival time in toxic solutions and direct measurements of respiratory rate under different physiological conditions in Planaria

PHYSIOLOGICAL CONDITION	SURVIVAL TIME	OXYGEN CONSUMPTION	CO_2 PRODUCTION
Age (size)	Shorter the smaller (younger) the worm	Greater the smaller the worm	Same as for oxygen consumption
Injury	Shorter in injury	Greater in injury	Same
Regeneration	Shorter in regeneration	Greater in regeneration	Same
High temperature	Shorter	Greater	Same
Low temperature	Longer	Less	Same
Movement	Shorter than in rest	Greater than in rest	Same
In acid solution	Longer	Less	
Different levels	Shorter in anterior levels	Greater in anterior levels	Same
Late starvation	Shorter	Greater	Same
Feeding	No change in body surface	Greater	Same
Early starvation	No change or shorter in body surface	Less	Same

oxidations," as Allen states. The method of determination of total metabolism in which Allen places such faith also fails to give any information about specific processes in the body unless other factors are controlled and eliminated. If one wants to measure the respiration in muscular work one cannot at the same time give the experimental animal a meal.

It may then be reiterated that as far as our experiments go, the survival time in toxic solutions is in simple animals a measure in general of the metabolic rate, as long as internal conditions remain constant. Survival time may not be an accurate measure of total respir-

TABLE 6

Detailed data on the oxygen consumption of Planaria maculata during starvation. Final calculation per unit weight per unit time in table 1

TIME SINCE FEEDING	LOT 1		LOT 2		LOT 3		LOT 4		LOT 10	
	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight
	cc.	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*
1 day	0.54 (1½ hrs.)	1.833			0.43 (1½ hrs.)	1.277				
	0.56				0.55					
4 days	0.43 (1½ hrs.)	1.667	0.54 (2 hrs.)	0.874	0.36 (1½ hrs.)	1.110	0.46 (2 hrs.)	1.104		
	0.43		0.47		0.36		0.49			
1 week	0.41 (1½ hrs.)	1.584			0.33 (1½ hrs.)	1.046				
	0.36				0.37					
2 weeks	0.42 (2 hrs.)	1.222	0.37 (2 hrs.)	0.634	0.42 (2 hrs.)	0.862	0.38 (2 hrs.)	0.818	0.36 (3 hrs.)	0.521
	0.36		0.34		0.36		0.36		0.35	
4 weeks	0.28 (2 hrs.)	0.764	0.23 (2 hrs.)	0.362	0.30 (2 hrs.)	0.579	0.31 (2 hrs.)	0.474	0.15 (3 hrs.)	0.205
	0.25		0.19		0.27		0.23		0.17	
6 weeks	0.29 (2½ hrs.)	0.507	0.19 (3 hrs.)	0.149	0.24 (2½ hrs.)	0.298	0.21 (3 hrs.)	0.215	0.19 (6 hrs.)	0.093
	0.28		0.15		0.22		0.19			
8 weeks	0.18 (3 hrs.)	0.258	0.11 (3 hrs.)	0.062	0.15 (3 hrs.)	0.166	0.14 (3 hrs.)	0.111		
	0.16		0.11		0.14		0.14			
10 weeks	0.08 (3 hrs.)	0.098			0.12 (3 hrs.)	0.065	0.16 (6 hrs.)	0.043		
	0.06				0.10					

TABLE 7

Detailed data on the oxygen consumption of Planaria agilis during starvation. Final calculation per unit weight per unit time in table 3

TIME SINCE FEEDING	LOT 5		LOT 6		LOT 7		LOT 8		LOT 9	
	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight
	cc.	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*
1 day	0.51 (1½ hrs.)	2.196	0.60 (1½ hrs.)	2.417	0.80 (2 hrs.)	1.632	0.84 (2 hrs.)	1.755	0.95 (2 hrs.)	2.062
	0.54		0.62		0.82		0.82		0.96	
4 days	0.39 (1½ hrs.)	1.730	0.48 (1½ hrs.)	2.175			0.46			
	0.38		0.56							
1 week	0.34 (1½ hrs.)*	1.500	0.50 (1½ hrs.)	2.031						
	0.27		0.39							
2 weeks	0.35 (2 hrs.)	1.329	0.50 (2 hrs.)	1.827	0.41 (2 hrs.)	1.022	0.46 (2 hrs.)	1.275	0.44 (2 hrs.)	1.188
	0.29		0.42		0.38		0.42		0.41	
4 weeks	0.37 (2 hrs.)	1.083	0.50 (2 hrs.)	1.469	0.31 (2 hrs.)	0.812	0.39 (2 hrs.)	1.052	0.46 (2 hrs.)	1.289†
	0.27		0.36		0.28		0.34		0.40	
6 weeks	0.27 (2½ hrs.)	0.871	0.41 (2½ hrs.)	1.018	0.32 (3 hrs.)	0.558	0.38 (3 hrs.)	0.725	0.42 (3 hrs.)	0.828
	0.28		0.36		0.32		0.34		0.38	
8 weeks	0.33 (3 hrs.)	0.667	0.43 (3 hrs.)	0.749	0.27 (3 hrs.)	0.394	0.29 (3 hrs.)	0.488	0.33 (3 hrs.)	0.557
	0.30		0.37		0.24		0.25		0.30	
10 weeks	0.25 (3 hrs.)	0.480	0.28 (3 hrs.)	0.510	0.19 (3 hrs.)	0.288	0.20 (3 hrs.)	0.346	0.21 (3 hrs.)	0.338
	0.24		0.25		0.21		0.22		0.25	
12 weeks	0.17 (3 hrs.)	0.322	0.20 (3 hrs.)	0.336	0.28 (5 hrs.)	0.173	0.29 (5 hrs.)	0.202	0.33 (5 hrs.)	0.248
			0.16							
16 weeks	0.15 (5 hrs.)	0.093	0.18 (5 hrs.)	0.143						

* The higher rate of oxygen consumption usually found in the first of each pair of determinations is due to movement. The amount of movement is greatest in the middle of the starvation period. It was later eliminated to some extent by placing the worms in the experimental flasks the day before the test.

† Worms of the same kind added.

atory rate because other factors than respiratory rate may be concerned in survival time. Argument over the reliability of the susceptibility method has now been rendered unnecessary because all of the main conclusions drawn by the use of this method have been verified by direct determinations of oxygen consumption and carbon-dioxide production.

SUMMARY

1. This paper is concerned with the rate of oxygen consumption during starvation of Planaria maculata and Planaria agilis and was undertaken in reply to a paper by Allen (1).

2. Contrary to the results of Allen it was found that in both of these species within the time limits specified in his paper, the rate of oxygen consumption is increased in the later periods of starvation. His data are criticised and it is pointed out that they are with one exception inadequate and unsatisfactory; the one exception contradicts his own conclusion.

3. Planaria agilis has a lower metabolic rate, greater food reserves, and loses weight more slowly in starvation than other species. For this reason it must be starved for a longer period before its rate of oxygen consumption increases.

4. Owing to the slow rate of starvation of Planaria agilis, there is a period from the second to the sixth week of starvation when the oxygen consumption is nearly constant. This is not the case in other species.

5. The susceptibility of Planaria agilis to potassium cyanide also fails to increase during starvation as rapidly as in other species. Contrary to the contentions of Allen, the susceptibility results in this species are then in general agreement with the results from direct measurement of the oxygen consumption.

6. Contrary to the statements of Allen, it was found that in Planaria agilis worms reduced to a small size by starvation have as high a rate as or a higher rate of oxygen consumption than small recently fed worms of the same size.

7. A general discussion of the utility of the susceptibility method as a measure of metabolic rate is given and it is pointed out that no discrepancies are at present known to us between the results by the susceptibility method and the results by direct determination of respiratory rate, except those concerned with feeding. In the case of feeding, it is necessary to draw a distinction between the metabolism and susceptibility of the digestive tract and those of the body wall.

8. The results in this paper further support the general conception maintained by Child for years that the metabolism of organisms is increased in starvation and that starvation is therefore a means of bringing about rejuvenescence.

BIBLIOGRAPHY

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VASOMOTOR REFLEXES FROM RECEPTOR STIMULATION IN INTACT ANIMALS

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Of recent years the investigation of vasomotor reflexes in mammals other than man has been carried on chiefly on the basis of artificial stimulation of nerve trunks, rather than by means of receptor stimulation; the older physiologists, on the other hand, gave much attention to the effects on blood pressure of excitations applied to the receptors themselves. The latter method would appear to be the more logical if the object sought is to determine the normal mode of functioning of the vasomotor apparatus, although where the underlying purpose is the study of reflex action, with the vasomotor reflexes selected as examples of such action, direct stimulation of sensory nerve trunks is not only valid, but has much to recommend it, both in theory and practice.

One of us (M.) has been for some years interested in vasomotor reflexes, not so much from the standpoint of their significance as regulating factors of the circulation as for the light they may throw on the reflex functioning of the nervous system itself ((1) to (6)). In all the experiments described in the above series of references the elicitation of reflex responses was by means of stimulation of nerve trunks. Certain of the findings therein have suggested the desirability of repeating some of the experiments of the older investigators on the reflex vasomotor effects of *receptor* stimulation, with the attention specially directed to the bearing of such experiments on theories of nervous action.

PROCEDURE

Our plan of study called for the elicitation of vasomotor reflexes in conscious and in very lightly narcotized animals, as well as in decerebrate and in deeply anesthetized individuals. It followed that the method of recording the responses must be applicable to conscious

animals. Blood pressure determinations were ruled out because of the operative procedures involved; the choice was thus restricted to plethysmographic methods, or to such as use temperature variations as indices of vasomotor changes. Either of these latter have the disadvantage of showing only local effects, as contrasted with the general influences revealed by blood pressure determinations. A vasomotor response that is restricted to the splanchnic region might escape detection completely under such circumstances. This is a limitation that is unavoidable, however, in experimentation on conscious animals. If the site of application of the recording device is fortunately chosen it should reveal any vasomotor change that affects cutaneous areas. One important desideratum is that the region selected shall be known to have both constrictor and dilator innervation, since interpretation of the interaction of dilator and constrictor responses is a significant feature of the general problem (5).

Following the example of Grützner and Heidenhain (7), our experiments were made on rabbits. The varieties were those widely raised in California for market purposes, including some common domestic rabbits and some Belgian hares. On the whole we found the latter less satisfactory than the smaller and hardier rabbits. We experimented at some length with the nasal cavity as a plethysmograph, using the method of Tschalussow (8) as modified by Mendenhall (9) but found it unsuited for work with rabbits. We then turned to the ear as a field of study. Since in rabbits the ears play an undoubted, and probably considerable, part in temperature regulation (10), they might be expected to be as sensitive to vasomotor influences as any of the cutaneous areas. That they have dilator as well as constrictor innervation appears from the work of Winkler (11), cited by Bayliss (10).

To record vasomotor changes in the ear we used an ordinary air plethysmograph, communicating with a sensitive tambour, whose lever wrote on a slow drum. The air plethysmograph offers a double advantage for work of this kind; in addition to being itself a sensitive recorder of volume changes in the ear, it responds also to changes of temperature; vasodilatation would bring about both an increased ear volume and a higher ear temperature; the latter by causing expansion of the air in the plethysmograph would add its effect to the increase of ear volume, enlarging the tracing on the drum. Obviously sudden changes in temperature due to external influences had to be guarded against, but this was a simple matter.

For keeping unanesthetized rabbits in position we used a board 6 inches wide, mounted on a pedestal about a foot high. This board was cut away at front and rear, so that when a rabbit was laid on it his legs would hang down comfortably. A couple of short straps were buckled around the board and over the back of the animal, and a rest provided for his chin. With this arrangement a rabbit would remain quiet for two or three hours, making few movements, except when stimulated thereto in connection with our experimentation. At first we tied the head firmly, but this proved unnecessary and since it often seemed to impede the blood flow through the ears was abandoned. A precisely similar arrangement was employed with anesthetized or decerebrate animals, except that with most of these we added a head-clamp. The hair was regularly clipped from all four legs, and from a considerable area of the back.

Stimulations. Tactile, thermal and auditory stimuli were used with success; visual stimuli, in the form of light flashed on and off before the eyes, gave negative results so far as vasomotor manifestations are concerned.

For tactile stimuli we followed the practice of Grützner and Heidenhain (loc. cit.) and used blowing upon the skin, rubbing the mouth, inside, and upon the lips, with a hard instrument, and pulling at individual hairs with forceps, all gentle stimuli and, according to the experience of those investigators, highly effective in evoking vasomotor reactions. We found the free ear the most favorable spot on which to blow.

Several sorts of thermal stimuli were used. Dipping a leg into water of selected temperature for a measured time, thirty seconds to two minutes, proved moderately satisfactory. The leg, as stated above, was clipped of hair, and was thoroughly greased with vaseline before dipping into water. We used a container holding a liter; the temperature of the relatively large volume would remain fairly steady during the period of immersion. The position of the animal on the holder permitted the bringing of the vessel of water up around the leg without disturbing the experiment. The range of temperatures employed will be given further along, in connection with the detailed account of the results of the experiments. A second method of thermal stimulation, of which we made much use, consisted of placing an electric light, backed by a conical reflector of ordinary type, a short distance above the back, from which, as noted above, the hair had been clipped. To insure that the rays from the light should not strike upon the plethysmograph

directly, and so warm the air within it, we interposed a double screen; this consisted, first, of a large sheet of cardboard placed upright between the rabbit's back and head, and cutting off from the whole head and its surroundings any direct rays from the light; and second, of a cylinder of sheet asbestos, about double the diameter of the plethysmograph, placed about it. A sensitive thermometer inserted between the asbestos cylinder and the plethysmograph showed that no change of temperature occurred upon turning on the light. Another sensitive thermometer was laid along the animal's back, with the bulb in close contact with the skin, to enable some idea to be formed of the rate of warming of the back, as well as the maximum temperature reached. Since it was probable that the temperature of the thermometer bulb would rise faster than that of the skin, where the bulb was freely exposed to the light, we protected the bulb, in some of the experiments, with a small bit of asbestos. This precaution was not wholly satisfactory, since it was now probable that the skin in contact with the thermometer bulb, being protected with asbestos, as well as by the bulb itself, would warm up more slowly than areas fully exposed to the light, and so introduce an error in the opposite direction. To cool the skin of the back we placed upon it a cloth bag of cracked ice, or obtained the desired effect by pouring a small quantity of ether upon it.

Various auditory stimuli were tried, but the only really satisfactory one consisted of a shrill whistle, blown near the ear of the rabbit, but with precaution not to allow the blast of air to strike the animal.

VASOMOTOR RESPONSES IN NORMAL RABBITS

For experiments on conscious animals we were obviously limited to stimuli which would be endured without struggling, since quiet was essential to the securing of a convincing record. This meant the confining of the stimulation, in general, to the levels below the threshold of pain, or the selection of stimuli which have no marked painful quality, even when intense. The investigation became, in a sense, a comparison of the thresholds of vasomotor and skeletal muscle reflexes, since our practice was to push the stimulation, whenever possible, until the animal began to struggle, observing meanwhile such changes in ear volume as occurred.

Tactile stimuli. Confirming the findings of Grützner and Heidenhain (7) that gentle stimulation is effective in arousing vasoconstriction, we obtained repeated shrinkage of ear volume by blowing with the

breath on the exposed ear, or the back (clipped of hair) or the hind leg, or the nose. The onset was prompt; the maximum effect was reached within fifteen seconds, and the return to former volume was completed within thirty to forty-five seconds. Contact of an ice pack with the back caused, on several occasions, an ear shrinkage that was obviously due to the contact and not to any lowering of temperature, since the onset was as prompt as in the blowing experiments, and recovery occurred as quickly. Localized irritation, as by pulling at a small group of hairs with forceps, did not produce any demonstrable change in ear volume.

A feature of these responses which we wish to stress is that they were definitely more pronounced early in the course of the experiments than later. There appeared to be a habituation or fatigue which operated to cut down their magnitude. To illustrate: a large black male rabbit, which had been experimented on on several previous occasions, gave, within the first five minutes of the experiment of February 19, 1920, typical responses to blowing upon the ear. Ten minutes later the response was still evocable, but much reduced; a half-hour later the same sort of stimulation failed to elicit any response. Throughout the period stimuli of one sort or another were applied every minute or two. The animal was then removed from the apparatus and allowed to run about the floor for ten minutes. It was then replaced in position for recording ear volume changes and similar stimuli applied. Shrinkage of ear volume occurred, comparable with that obtained at the very beginning of the experiment; again, however, there was a diminution of effect, so that at the end of fifteen minutes the response was no longer obtainable.

Auditory stimuli. Shrill whistling, prolonged for five or six seconds, brought about ear shrinkage very much like that obtained from tactile stimulation. The latency was short, and the duration about equal to that of the responses to blowing on the ear or back. To assure ourselves that the effect on the plethysmograph was actually due to a change of ear volume, and not to a movement of the tympanic membrane, we stuffed the external auditory meatus with vaseline; this procedure did not change the response. These observations are in accord with those of Dogiel (12). We observed a habituation to auditory stimulation similar to that reported above for tactile. The vasoconstriction from whistling was frequently greater at first than the maximum from blowing on the back or ear, but quickly became less,

and complete failure occurred earlier. A short rest outside the apparatus restored the effectiveness of the stimulation momentarily.

Thermal stimuli. We attempted to lower the temperature of the skin of the back by laying an ice pack on it and by pouring ether on it. In spite of repeated attempts the first method failed to yield demonstrable vasoconstriction in conscious rabbits. The animal would endure the presence of the ice pack for about two minutes, but would then begin to struggle so violently that the pack had to be removed. No change in ear volume occurred during the two minutes in which the pack was in contact with the back, except for the transitory vasoconstriction due to contact described above. The chief interest of this observation lies in the apparent demonstration that cooling of the back becomes sufficiently uncomfortable to arouse the conscious animal to definite efforts at escape before the stimulation of cold reaches the point of evoking demonstrable vasoconstriction. We were uniformly successful in obtaining vasoconstriction when ether was poured on the back. The effect developed promptly, reached a maximum within a half-minute, and usually passed off within two or three minutes. A small volume of liquid, roughly 1 cc., gave the best results. On one occasion we tried pouring about 5 cc. on the back. Vasoconstriction began to develop in the usual way, but before it had attained maximal extent the animal displayed signs of annoyance at the odor, and presently began to struggle. We attribute the constrictor effect induced by ether to the cooling brought about by the rapid evaporation, and we have evidence from the thermometer placed against the back that there was prompt and marked cooling; on two occasions the back temperature fell eight degrees in two minutes. The possibility that the ether may have had an irritating effect apart from the cooling is not excluded, although no marked irritation is experienced from contact of ether with the human skin. It is possible that our superior success with ether as compared with the ice pack was due to a degree of fright induced by the strange odor, which kept the animal quiet except in the case where an excessive dose was used, when the desire to escape became paramount.

Vasodilatation (swelling of ear) was readily obtained in conscious rabbits by warming the back with an electric light in the manner described above. The animals usually endured the warming for several minutes without showing signs of discomfort. In every trial but one of our series vasodilatation began to show itself before any skeletal muscle move-

ments were made. In the one exceptional case there were signs of restlessness before vasodilatation became perceptible, but the movements were not extensive enough to necessitate discontinuance of the experiment, and increase in ear volume began to show itself before struggling became pronounced. There was considerable variation in the latency of the vasodilatation; in some cases the ear began to swell within a few seconds after turning on the light; in others two or three minutes elapsed before visible increase in ear volume appeared. In the exceptional case mentioned above, in which struggling preceded ear swelling, the warming continued for seven minutes before the bodily movements began; the swelling of the ear did not become demonstrable until eleven minutes after applying the electric light. As stated in a former paragraph, we do not consider the recorded changes in back temperature very significant because of the large probable error, inherent in the use of a mercury thermometer laid in contact with the skin. It is, however, perhaps worth noting that on four occasions the rise in skin temperature preceding the beginning of struggling on the part of the animal coincided at 6°.

VASOMOTOR REFLEXES IN DECEREBRATED RABBITS

Our method for preparing the rabbits was that usually employed in experiments involving the procedure of decerebration. Light ether anesthesia was maintained throughout the operation up to the completing of the cut across the brain stem. Clamps were applied to the carotids, and the vertebral arteries were occluded by pressure of the fingers and thumbs of an assistant at the sides of the vertebral column immediately behind the skull. In rabbits stoppage of the circulation through the vertebrals is easily accomplished in this manner. As soon as possible after completion of decerebration the pressure on the vertebrals was released and the clamps on the carotids removed.

Tactile stimuli. The only suggestions of vasoconstrictor reflexes following tactile stimulation were obtained within the first fifteen minutes after decerebration. On several occasions we noted what looked like extremely slight positive responses, but only once was there a reaction that at all approached in magnitude our usual responses to similar stimulation in normal rabbits. On this occasion the reaction followed laying the hand gently on the rabbit's head. About a minute later repetition of the stimulus was followed by vigorous galloping movements on the part of the animal. Perhaps it is worth noting that

whenever our procedures aroused decerebrate rabbits to violent movements they took the form of galloping; this contrasts sharply with the struggling of conscious rabbits, whose effort was invariably to push themselves backward off the board on which they lay; an effort explicable when one recalls that the chief unaccustomed factor in the situation was the plethysmograph tube on the ear. In no case did tactile stimuli applied during the later stages of an experiment give positive vasomotor results. That this failure was not due to diminished sensitivity of the animals was shown by the presence during the same stage of the experiment of definite skeletal muscle responses to slight stimuli.

Auditory stimuli. We were unable to obtain a definite vasomotor reaction to shrill whistling in any instance, although skeletal muscle responses were readily elicitable in nearly all our trials. In one case the galloping activity, noted above as characteristic of violent effort in decerebrate rabbits, followed an auditory stimulus, although in most of our trials only slight movements of ear or head were noted. We wish to stress the point that two sorts of gentle stimulation, tactile and auditory, which in normal animals typically bring about well-marked vasomotor reflexes, fail almost completely in this regard in decerebrates, although skeletal muscle reflexes are obtained about as well in one form as in the other.

Thermal stimuli. We obtained typical vasoconstriction from pouring ether on the back of decerebrated rabbits; in appearance this corresponded fully with the response of normal animals to similar stimulation. Warming the back by means of an electric light gave vasodilatation (ear swelling) in all our trials except two. There appeared to be less tendency than in normal rabbits toward struggling; at least the animal would usually remain quiet for a longer time after the light was turned on than would the normals. On two occasions no ear swelling was manifest; on one of these there appeared to be a slight ear shrinkage. We carried out an extensive series of observations on the results of immersing a leg in water. As stated above, the leg was thoroughly greased with vaseline before immersion. A hind leg was used in most of our experiments. The front leg was tried, but in no case with positive results. Water at 1°C. (ice water) was used repeatedly but neither with decerebrates nor with rabbits in any other state did we ever obtain a perceptible vasomotor change therewith. For the tests with warm water temperature intervals of 5°C. were used, beginning with 35°. No changes in ear volume were observed at temperatures below 50°. At 50° vasodilatation occurred regularly; at 55° vasodilata-

tion occurred in approximately half the trials and vasoconstriction in the other half; at 60° and 65° only shrinkage in ear volume was seen, except that on one occasion a slight increase in ear volume preceded the constriction. These observations suggest that there is a temperature point at which reversal of vasomotor effect occurs.

VASOMOTOR REFLEXES IN RABBITS UNDER LIGHT ETHER ANESTHESIA

In our experiments in which ether was used as the anesthetic pains were taken to keep the narcosis as shallow as possible; some variation in depth of anesthesia was inevitable with the method of administration, which was by an ordinary nose cone. *Tactile stimuli* appeared to be less effective in eliciting vasomotor responses than in unanesthetized rabbits; at least such as we tried were ineffective, although they were pushed to the point of inducing struggling. Shrill whistling was followed by transient shrinkage of the ear; very definitely in the early stages of the experiment; less so in the later stages. It was our impression that there was a habituation to the stimulus, similar to that suggested by the experiments on conscious animals, but the possibility that increasing depth of narcosis might account for the lessened effect is not excluded.

Warming the back by means of an electric light resulted in vasodilatation; with very light narcosis the animal would endure the warming without struggling for only a few minutes, so that extended exposure to the warming could not be studied. Immersing a hind leg in water at 45°C. was followed in one instance by definite ear swelling, although shortly afterward temperatures of 49°, 50° and 51° gave negative results. Water at 52° brought about commencing vasodilatation, but almost coincident with the beginning of the change in ear volume the animal became restless and the hot water had to be withdrawn. When the leg was immersed in water at 55° the animal struggled so promptly and violently that no vasomotor record could be obtained.

VASOMOTOR REFLEXES IN RABBITS PARTIALLY ANESTHETIZED WITH URETHANE

We were much interested in the possible effects of urethane on the responses to receptor stimulation, since Martin and Lacey (1) had shown that the threshold of vasomotor reflexes from *nerve*-trunk stimulation is not very much raised by urethane. We performed our ex-

periments with urethane in two series; in the first only half the standard dosage was used (1 gram per kilo body weight); in the second we used standard full dosage. The appearance of rabbits that have received only half the usual dose of the narcotic differs characteristically from that presented by fully anesthetized animals. They show a degree of prostration, but chiefly in the rear half of the body. The hind legs are frequently quite useless, but some control is retained over the front legs, and the neck muscles appear to be only slightly affected so that the head is held in about the usual position. This is in sharp contrast to the complete muscular relaxation of rabbits under the influence of full dosage.

Tactile stimuli. The same kinds of tactile stimuli were used as under the other experimental conditions employed in the investigation, namely, blowing on or pinching the ear, blowing on the back or leg, rubbing the inside of the mouth with a blunt instrument. In about half the trials ear volume showed slight decrease, indicating some vasoconstriction; in the others no change was seen. On two occasions pinching the ear brought about struggling, showing that skeletal muscle reflexes were rather readily evocable.

Auditory stimuli. In only one out of nine trials did whistling fail to elicit vasoconstriction; one of the results recorded as positive was so slight as to be questionable; all the others were definite.

Thermal stimuli. Cooling the back by pouring ether on it did not give as satisfactory results as in normal or decerebrated rabbits. We did not obtain in any instance as good a record of ear shrinkage from this procedure as appeared regularly in those series. The partially urethanized animals spoiled the record by struggling much more frequently, but in the few cases in which struggling did not occur clear-cut vasoconstriction was not manifested, although the record indicated its presence to a very slight degree.

Warming the back with an electric light was followed regularly by well-marked vasodilatation. The warming was ordinarily endured for only about four minutes; at the end of that time struggling would begin and the light have to be removed. There would have been time, meanwhile, for the ear swelling to show itself. Immersing a hind leg in warm water gave vasodilatation at 45°, 50°, and 52°; at the latter temperature the effect was very slight; possibly there was a habituation from the previous immersions at the lower temperatures. At 54° the animal struggled thirty seconds after applying the warm water; no swelling of the ear had developed at that time.

Observations on the late effects of urethane. We were interested in the late effects of urethane on the relative sensitiveness of the vasomotor and skeletal muscle reflex paths; accordingly we reëxamined our animals at the end of twenty-four hours, and in one case of a half-dose animal again after forty-eight, and after seventy-two hours. Our rabbits that had received only half the usual urethane dosage were in general appearance nearly over the effects of the drug on the day following administration. They ordinarily showed some lack of coördination in the movements of the hind legs, but in other respects behaved much like normal rabbits. Such skeletal muscle reflexes as attended our receptor stimulations were definitely more easily elicited than on the preceding day. In contrast to this near recovery of skeletal muscle control is the interesting fact that vasomotor reflexes were nearly inelicitable. Vasodilatation resulted from warming the back with an electric light, but all our other procedures were negative, except that in a single instance rubbing the inside of the mouth was followed by a minute curve in the tracing which may have been an indication of very slight vasoconstriction.

After forty-eight hours the single rabbit that was observed extensively presented, in all respects, the appearance of complete recovery. It responded to mechanical and auditory stimulation by vasoconstriction in precisely the fashion of normal rabbits. The only difference that we observed was that no ear shrinkage followed the pouring of ether on the back. To determine whether this response would be reëstablished we repeated the tests on the following day, namely, seventy-two hours after narcotization. In addition to positive results from all our other tests we now obtained definite vasoconstriction when ether was poured on the back.

VASOMOTOR REFLEXES IN RABBITS FULLY ANESTHETIZED WITH URETHANE

As stated above, rabbits under full urethane narcosis show complete muscular relaxation. In our experience this comes on quite promptly; it is accompanied by a pronounced slowing of the breathing. We made a number of counts of the respiratory rate before and after administering urethane, and obtained slowing from a preliminary rate ranging between 140 and 180 a minute to a rate of 60 to 70 a minute.

Tactile stimuli. We obtained definite changes in ear volume following tactile stimulation in about 70 per cent of our trials; since we experimented with various means of stimulation the percentage of nega-

tive results is probably not unduly large. Contrary to our experience with rabbits in other states as regards narcotization, in all of which mechanical stimulation gave only vasoconstriction, we observed in nearly a third of our positive trials in this series swelling of the ear (vasodilatation); in the remaining two-thirds the usual vasoconstriction was seen. In an attempt to account for the occurrence of contrary responses from similar stimulations we classified the tactile stimuli employed as gentle or harsh. To the first group were assigned stroking the skin, handling the ear gently, and blowing on the ear or back; in the second were included pinching the skin hard with fingers or forceps and rubbing the inside of the mouth with a blunt instrument; the free ear was the area most frequently selected for pinching. All our vasodilator responses but one resulted from the application of stimuli classified as gentle; 70 per cent of the constrictor responses, on the other hand, followed harsh stimulation.

Notwithstanding the complete muscular relaxation characteristic of fully urethanized rabbits skeletal muscle reflexes were not wholly absent. On three occasions mechanical stimulations were followed by struggling. Two of these were after harsh stimuli; the third occurred after blowing on the skin, a stimulus classified by us as gentle.

Auditory stimuli. Shrill whistling was followed by ear shrinkage (vasoconstriction) regularly in the early stages of the experiments. In only three early trials did we fail to get the response. In all these experiments stimulation was begun as soon as possible after signs of complete narcotization had developed. In only one case did we observe a positive effect as much as forty minutes after the beginning of experimentation. In general urethanized rabbits seemed to respond positively to auditory stimulation only during the very early stages of narcosis. In all our experiments of this series good responses to tactile stimuli were elicitable a half-hour or more after auditory stimulation had ceased to be effective. In two instances transient ear swelling followed the whistling; these were the only cases in our entire experience in which any other positive response than vasoconstriction followed auditory stimulation.

Thermal stimuli. Both our methods of lowering skin temperature, laying an ice pack on the back, and pouring ether thereon, brought about vasoconstriction repeatedly. We failed to get this result only twice, both times during the very late stages of the experiments. Raising the back temperature by means of an electric light was followed

just as regularly by swelling of the ear (vasodilatation). In several instances the onset of the dilator reaction was considerably delayed. During this latent period we observed several times, but not invariably, a slight but perfectly definite ear shrinkage, which suggests the possibility of a local vasodilatation in the warmed area of the back which would cause an increased flow of blood through that region at the expense of other parts of the body. With the development of general reflex vasodilatation active swelling would succeed this passive shrinkage in such areas as the ear. On only two occasions did the warmth on the back lead to struggling. This contrasts sharply with the invariable occurrence of this reaction in normal rabbits.

Immersing one hind leg in warm water was followed by swelling of the ear (vasodilatation) in just half our trials; all the others were negative except two, in which there appeared to be a slight shrinkage of the ear. Positive vasodilatations were obtained at temperatures of 50° and upward; none were seen at 45°, although that temperature was tried repeatedly. We obtained in two cases ear swelling at 65°; subsequent tests at 70° and 75° were negative. We have reason to suspect that injury to the receptors occurred at 65°, since the leg that had been immersed in this very hot water was much swollen and blistered on the following day. For comparison it might be well to note that water at 50° to 52° is extremely painful to the human hand if immersion is maintained for as much as a minute, as we demonstrated upon ourselves. The observations which we interpreted as showing vasoconstriction occurred at 53° and 58°. There was struggling, sometimes violent, at nearly all temperatures above 53°. The latency was sufficient to allow the change in ear volume to show itself while the animal was still quiet. In one case the skeletal-muscle response to immersion of a hind leg in hot water took the form of definite running movements.

Late effects of full urethane narcotization. In an earlier section we described observations on the recovery from half doses of urethane. Similar observations were made on the late effects of full doses. At the end of twenty-four hours we found substantially the same situation with respect to vasomotor responsiveness that is recorded above for partially urethanized animals, namely, an almost complete insensitiveness. We have one observation of ear swelling following warming the back with an electric light, but with by far the longest latent period (thirteen minutes) seen in the investigation. We have also one record of vasoconstriction resulting from pouring ether on the back. All our

other numerous tests, including all the kinds described in this paper, were negative. These rabbits showed much less progress toward recovery of skeletal muscle control than did those under half-urethane after a similar interval. They did, however, respond regularly by struggling to most forms of mechanical stimulation, to ether on the back, and to immersion of a hind leg in water at temperatures above 51°C. The respiratory rate was that of complete urethane narcosis, namely, 60 to 70 a minute.

A single rabbit that was examined at intervals until completely recovered gave the following history: at thirty hours after administration of urethane the breathing was still slow, 72 in the minute; there were no well-marked ear volume changes as the result of stimulation, although very small curves appeared in the tracings in connection with the application of some of the stimuli. An interesting feature of such as did appear was that with one exception they were of opposite sign to the usual responses; thus, both tactile and auditory stimuli gave what would have been interpreted as vasodilatation if the curves had been more pronounced, whereas the ordinary effect of such stimuli is definite vasoconstriction. One tactile stimulus did give apparent ear shrinkage, this being the single exception mentioned above. After forty-six hours the breathing in this rabbit had returned to the normal rate, 170 in the minute; there was still considerable muscular incoördination. The vasomotor responses to stimulation were more marked than after thirty hours, but again both auditory and tactile stimuli were followed by dilatation instead of by the usual constriction. Warming the back with an electric light gave dilatation; immersing a hind foot in warm water (45°–55°) was without effect on ear volume; at 55° the animal struggled. Fifty-four hours after anesthetization both tactile and auditory stimuli yielded vasoconstrictor reactions, although in five out of eight trials there was a slight preliminary dilatation preceding the constriction. Pouring ether on the back resulted at this time in the usual constrictor response. At the end of seventy-six hours the vasomotor reactions were in every respect similar to those previously obtained by us from unanesthetized rabbits.

DISCUSSION

The transient vasoconstrictor response obtained by us under mild tactile stimulation appears to be identical with that described by Grützner and Heidenhain (7) for a similar situation. The investigators just

mentioned observed a marked heightening of the response under curare poisoning, and were so impressed therewith that their further study concerned itself with the reflex as exhibited by rabbits in curare paralysis, to the exclusion of its consideration in unpoisoned animals. In addition to their finding that the vasoconstriction was heightened in curare paralysis they showed that very severe stimulation of the cutaneous endings, instead of affording even more pronounced results, failed to induce any response whatever. Before discussing the features in which our observations agree with those of Grützner and Heidenhain a brief consideration of these curare effects seems desirable. The point about them which was chiefly stressed by the authors was that they appeared only at a certain dosage of curare, a fact which probably explains the failure of recent workers in the same field (Sollmann and Pilcher (13), Martin and Stiles (3)) to report the phenomena. To our mind this confinement of the effect to a certain stage of curare poisoning signifies an acute disturbance of nervous equilibrium due to the drug, and negatives the application of the observations to the interpretation of normal nervous functioning. We are not willing to accept, for this reason, the view of Grützner and Heidenhain that the marked rise of blood pressure seen in their curare experiments is a mere accentuation of the slighter rise obtained from similar stimulation in normal animals. They consider the possibility that the action on the vasoconstrictor may be a secondary result of primary psychic excitation, and reject it on the ground of the finding that the curare rise of pressure persists after cutting across the brain-stem. Dogiel (cited by Luciani, 12) found that normal animals showed vasoconstriction in response to auditory stimulation, and that the response disappeared under curare. We confirm the finding so far as normals are concerned. In the section of this paper dealing with reflexes in decerebrate animals we have reported our virtual failure to obtain vasoconstriction from either tactile or auditory stimulation in uncurarized decerebrate rabbits. We incline, therefore, to the view that both in our experiments and in those of Grützner and Heidenhain and of Dogiel the transient vasoconstriction seen in normal animals was due to a secondary influence on the vasomotor mechanism following psychic excitation. This interpretation places the reaction in precisely the same category with the familiar plethysmographic findings in man, wherein mental alertness is accompanied by arm shrinkage. In our opinion this view is strengthened by our observation that there is an apparent habituation to the stimulus. This is just what one would expect should follow repetition of a stimulus

which depends for its effect on psychic excitation, particularly when the stimulation itself is of so mild a character as was used in this connection. The confinement of our positive results to the very early stages of urethane and ether anesthesia agrees also with this conception. By way of further confirmation we have the finding of Jacobson (14) that localized skin stimulation in human beings is unproductive of vasoconstriction through the operation of a direct reflex, although, as is well known, definite vasoconstriction occurs in man whenever mental alertness is elicited, however slight the exciting agent. The discussion up to this point summarizes into the generalization that mild tactile or auditory stimulation is productive of vasoconstriction only when it operates to bring about psychic excitation, which latter is the immediate cause of the vasomotor activity.

In addition to the transient vasoconstriction just discussed we obtained a similar, but more prolonged, reaction when an area of skin was chilled. The significant combination of influences here appears to be a physiologically intense stimulus (lowering the back temperature 8 degrees in two minutes) and a widespread area over which the stimulation is applied. That this combination is highly disturbing is shown by the prompt objection made by unanesthetized animals to an ice pack on the back. The cooling by pouring ether on the back was endured, but only, in our opinion, on account of fright induced by the odor of the ether. Deeply anesthetized and decerebrated animals gave the reaction regularly, showing that it is not dependent on psychic excitation.

Our most prominent exhibitions of vasodilatation occurred as the result of applying warmth to the skin, and of the two methods employed the more striking as well as the more constant was that which more nearly approached the normal conditions of the environment, namely, the gradual warming of a large area of the back by means of an electric light. There is nothing in our experiments to show whether this dilatation is reflex or is due to a direct effect of warming the blood, but the experiments of Winkler (cited by Bayliss, 10) gave evidence that a similar manifestation was definitely of reflex origin. A feature we wish to emphasize in connection with this reaction is that it occurs in response to a very gentle type of stimulation. If we may judge by the behavior of conscious animals, no disturbing element was contained in the back warming until it had been in operation for a number of minutes. Our other method of eliciting vasodilatation, by dipping a leg into water, gave much less uniform results. The sensory area in-

volved was relatively limited, and the range of stimulation-strength which would evoke the reflex was narrow; cold water and water of moderate warmth gave negative results; water too hot aroused skeletal-muscle activities which nullified the observations. In several cases the response was reversed; this occurred usually, but not invariably, at the highest temperatures that could be used satisfactorily. The suggestion is that the attainment of water temperatures of definitely painful character tends toward the vasomotor response usually associated with pain, namely, vasoconstriction, while the gentler stimulation of warmth tends rather to induce vasodilatation. In none of our experiments with thermal stimuli was there any indication that psychic excitation is concerned in the reaction. The experiments of this group, while harmonizing on the whole with the accepted view that the specific effect of warmth stimulation is vasodilatation, and of cold vasoconstriction, seem to us also susceptible of explanation in terms of the volume of nervous discharge set up. That is, that gentle or restricted stimulation, evoking only a moderate discharge, induces typically vasodilatation, while intense or widespread stimulation, which sets up a considerable volume of nervous impulses, tends to arouse vasoconstriction. It will be seen that this is an attempt to avoid relating particular vasomotor reactions to the stimulation of specific sense organs, and to assign them rather to certain volumes of nervous discharge. In a subsequent communication additional evidence to this effect will be adduced, and a more detailed consideration of the bearing of this conception on the whole problem of reflex conduction presented.

SUMMARY

Experiments on the effects on ear volume of tactile, auditory and thermal stimulation in normal, decerebrate and narcotized rabbits lead to the conclusion that when vasoconstriction follows mild stimulation it is to be interpreted as a secondary effect, following primary psychic excitation. Gentle or limited stimulation not arousing mental alertness typically induces vasodilatation. Reflex vasoconstriction, not dependent on psychic excitation, requires intense or widespread stimulation for its elicitation.

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STUDIES IN PLACENTAL PERMEABILITY

I. The Differential Resistance to Certain Solutions Offered by the Placenta in the Cat

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The great development of chemistry in its relation to vital phenomena during the past twenty years has made imperative a more accurate and comprehensive examination of the various problems concerned with the permeability of the placenta. The many forces controlling the passage of substances into cells and through cellular membranes have become much better understood, and since the placenta can be considered as a cellular membrane separating the maternal circulation from the fetal, these advanced conceptions are available for application in the study of its permeability, so long recognized as of great importance in the physiology of the fetus. The greatest proportion of all workers in this field have approached the study of placental transmission by administering substances which might be qualitatively recognized in the fetus, and have merely stated whether or not they passed. Some have indicated the duration of the experiment, while others seemed to have considered the recognition of the substances as sufficient. Much of this work will, however, prove of permanent value because the large number of substances that have been used will permit of various classifications, and thus indicate further lines of investigation.

Nicloux (11) has made use of this opportunity, and has divided the substances into two groups, as follows: *a*, Substances soluble in water and diffusible, crystalloids; these traverse the placenta. *b*, Substances insoluble in water and not diffusible, colloids; these do not traverse the placenta. He has also (12) studied very accurately the transmission of ethyl alcohol administered to pregnant dogs and guinea pigs, and has found that in about one hour and thirty minutes after the introduction an equilibrium has been reached between the

alcoholic content of the maternal and fetal bloods. Combining the results of his own experiments with his analysis of the work of others, he concludes that while many other factors should be considered simple dialysis is the most important.

As one considers the question of approach to a further study of this most extensive and complex subject, another classification seems advisable, in order to indicate the possible methods which may be utilized. All substances coming into contact with the placental membrane on the maternal side must fall into one of two great classes, those normally present in the circulation and those which reach it accidentally. It is obvious that the former will meet with an established mechanism which is adapted to furnish constantly some given type of activity, varying with the substance under consideration. On the other hand the large number of foreign substances which have been studied in one way or another, and the still larger number which we can easily conceive of obtaining adventitious entrance to the maternal circulation, must of necessity meet a very different condition because of the lack of any specific adaptation on the part of the membrane. Any study of foreign substances must be secondary to the search after the primordial reactions of the placenta to normal constituents of the maternal blood, but nevertheless these reactions may be often studied to advantage by methods which utilize such adventitious materials.

From the standpoint of the physical laws which substances obey in their relations to membranes they obviously fall into the following groups: gases, liquids, crystalloids and colloids. Nicloux (13), in reporting elaborate observations on carbon monoxide, concludes that this gas, as well as oxygen and carbon dioxide, follows exactly the laws of gaseous diffusion in traversing the placenta. His work on alcohol has been referred to; his studies on chloroform and ether are of equal interest and importance. In the study of crystalloids there has been no work comparable to Nicloux's on gases and liquids. Among the better contributions on the relation of colloids to the placenta are those of Wertheimer and Delezenne (19) on peptones, Goldman (8) on acid-azo-dyes, Ascoli (1) on albumens, and Wertheimer and Meyer (20) on methemaglobin.

It is evident from this brief and incomplete outline of the work that has been done that the point of optimum attack for further investigations must be in the study of fluid and salt interchange between the mother and fetus. The information obtained from a careful investigation of this mechanism, with any method, must be of great importance

both directly and in the study of the more complex activities of the placenta in relation to the metabolism of proteins, fats and carbohydrates. The most fruitful method in the study of the normal pathways of fluid drainage is that proposed by Weed (17), and used by him to demonstrate the route followed by the cerebrospinal fluid in draining from the subarachnoidal spaces into the great sinuses. He injected a solution containing 0.5 per cent each of potassium ferrocyanide and iron ammonium citrate into the subarachnoidal spaces, and precipitated the Prussian blue in the course of its drainage through the arachnoidal villi. Later Shipley and Cunningham (16) demonstrated the passage of fluid into the veins and capillaries of the omentum by withdrawing that structure from the peritoneal cavity through a midline incision, and immersing it in a similar solution. Many other investigators have made use of the Prussian blue reaction, but have not used the balanced solutions for the study of fluid pathways. It is essential that these solutions be practically balanced to avoid the formation of soluble Prussian blue during the process of fixation. Both these salts pass readily through blood vessel walls, through arachnoidal villi, through pectinate villi and through the kidney. In each of these cases it is probable, if not certain, that they follow the normal direction of fluid passage.

When projected, the study of these salts in their relation to placental permeability was intended to analyze, if possible, any differential adaptation of parts of that structure to the passage of water and salts. In the case of these salts we are dealing with substances which are foreign to the organism, but which offer the advantage of being easily followed, not only to their ultimate location, but in the route traversed. A study of the transmission of salts normally present in the maternal organism would be of more value if they could only be followed in their course through the placenta. If by this method any information can be gained regarding salt and fluid interchange, this may be applied later to considerations concerning the normal salt constituents of the blood. Starting with the assumption that fluids pass easily from mother to fetus it was hoped that the cellular mediation could be analyzed in the same way as in the cerebrospinal system, the eye and in the peritoneum. It was a priori thought that both sodium ferrocyanide and iron ammonium citrate would easily traverse the placenta, because comparable salts had been shown to do so with ease by previous workers. Bar (2) described experiments in which potassium ferrocyanide was injected into the uterine veins of rabbits,

and was found after thirty minutes in the amniotic fluid. If then the potassium salt passes with such ease, it seemed probable that the resistance would not be greater to the less toxic sodium ferrocyanide. No such comparison was possible in the case of the iron ammonium citrate. However, certain organic salts have been found to traverse the placenta; Porak (14) showed that potassium acetate passed in experiments on rabbits, Zweifel (21) used sodium salicylate, administered to women in labor, Fehling (7) used sodium salicylate on rabbits, Launois and Briau (10) used this same substance on guinea pigs and rabbits, and Savory (15) used strychnine acetate on cats, dogs and rabbits. It is only by observing the effects of many different salts that we may determine any of the laws governing the actual interrelation of salts and tissues. In the placenta it is well known that the acid-azo-dyes and colloidal metals, as well as many other colloidal sols will not pass from the maternal to the fetal circulations. From this it is clear that there is a definite resistance offered to a group of substances whose molecules are large but which have, as far as we know at present, no other characteristic in common. These substances will all pass through capillary walls, through the lining of the peritoneum and through the kidney; and they will diffuse into certain cells in the body while other cells remain impermeable to them. On the other hand, capillary endothelium appears to be relatively impermeable to proteins, sometimes completely so, while easily permeable to the substances named above. Relative permeability is then a certainty both in tissue cells and in those cells functioning as membranes separating body fluids.

The experimental animals used in the past for these investigations on placental permeability have been confined almost entirely to rats, guinea pigs and rabbits. A very few workers have used dogs, cats and sheep; while many have performed experiments on women in labor, but few comparative studies have been made using the same substance in different species of animals. Bremer (5) calls attention to this in discussing anatomical characteristics of various placentae, and suggests that many of the conflicting statements in the literature can be explained by the variations in the animals used, and that more progress could be made by using a greater variety of species in the study of any given substance. He has studied the placentae in a large group of animals, in relation to the problem of fetal excretion, and therefore in relation to other organs which may assume all, or at least part, of the excretory function: the pronephros, the Wolffian body and the kidney. He says:

Mammalian embryos may be divided into two classes; those which retain functional Wolffian bodies until the kidneys are sufficiently developed to excrete urine, as is the case in birds and reptiles, and those in which the Wolffian bodies degenerate before the kidneys reach functional ability. The first class includes the pig, sheep and cat; the second the rabbit, guinea pig, man and rat. . . . . In those animals without the possibility of a continuous urinary excretion within the embryo, i.e., with an early degeneration of the Wolffian body, the placenta is provided with an apparatus similar to that found in the glomeruli of the Wolffian body or the kidney, thin plates of epithelium overlying the fetal capillaries. These appear in the placenta at about the time when the Wolffian body commences to degenerate, or in the case of the rat, which never develops mesonephric glomeruli, at about the time of the normal development of the glomeruli in other embryos. These plates continue and increase in number till term. They are apparently of greater extent in animals whose embryos are provided with large Wolffian bodies. In the placentae of those animals with a continuous embryonic urinary excretion, similar plates are not found, whether the placentae be of the opposed or conjoined type. From these facts it appears that embryonic and fetal urinary excretion takes place wholly through the placenta in the rat, at first through the Wolffian body and later through the placenta in the rabbit, guinea pig and man, but never through the placenta in the pig, sheep or cat. A knowledge of these differences should lead to more intelligent experiments on the permeability of the placenta.

Bremer does not discuss the question of differential permeability, but his observations are extremely interesting and important from a physiological point of view, and particularly emphasize the absolute necessity of studying all problems of embryological physiology from a comparative viewpoint, as variation in species may be sufficient to produce very conflicting results. Bremer's theory, founded entirely upon anatomical observations, has received but very little real physiological examination; it is, however, supported by the previous results obtained by Krunkenberg (9) who found that substances which did not pass through the placenta in dogs and cats, but were retained by the fetus, found their way easily into the maternal circulation in the case of guinea pigs and rabbits. Savory (15), on the other hand, described experiments which are contradictory. He found that strychnine acetate when introduced into canine fetuses caused convulsions in the mother in nine minutes and death in twenty-eight. His experiments on cats and rabbits gave the same results. Bremer considers: ". . . . that these modified plates have apparently become inactive membranes, through which a purely physical osmosis may take place, but which themselves may be supposed to be physiologically inert." While he applies his observations to excretion only, there seems to be no reason to preclude that the permeability is equal in both directions

for any membrane which is physiologically inert. Therefore experiments dealing with maternal to fetal transmission in animals having plates must be considered equivalent to a similar experiment in the opposite direction.

Goldman (8) has shown that trypan blue will not pass the placental barrier in the rat, but is easily excreted by the maternal kidneys. In some unpublished experiments I have found that trypan blue introduced into the circulation of rabbit fetuses in a late stage of gestation, was excreted by the fetal kidneys, but did not pass through the placenta into the maternal circulation. These experiments have also been performed on the fetuses of cats with the same results. It is obvious at once that the plates of the renal glomerulus must be different in their permeability from those in the placenta, when measured by this one class of colloidal sols. This does not in any way invalidate Bremer's conclusions that these placental plates indicate the route of fetal excretion and are physiologically inert, but prevents accepting the plates found in the placenta as an exact equivalent of those in the kidney.

Admitting Bremer's hypothesis to be substantiated, only one phase of the general problem will be settled because even if certain species of animals have specialized membranes which permit the passage of substances excreted by the fetus, still there is the essential necessity of the fetus receiving salts, water and foodstuffs from the mother, and these must pass the placenta irrespective of any variation in species. The problems still unanswered regarding the permeability of the placenta are innumerable. They are of vital interest both as regards their local and direct significance and in their bearing on phenomena elsewhere in the organism. They range from the general problem of cell permeability and the resistance of membranes, to special ability of the placenta as an organ to select, change and even synthesize substances needed in the fetal metabolism. The placenta is a single structure which probably exercises the combined functions of many types of cells, and therefore any laws which may be found governing its activities will undoubtedly be applicable in part to other cells. But the placenta differs so essentially in function from any other single tissue that even those laws having the most widespread application will probably have special significance here. As far as is possible all the laws developed for single cells and simple membranes should be applied to the special case of this complex and more primitive structure.

The present communication is the first of a series projected, and partly under way, having as their object the analysis of some of the more fundamental problems relating to placental permeability.

The plan has been to first analyze, by whatever methods are available, the normal fluid and salt interchange and to determine if possible what forces are most active in these processes. Then to determine as far as possible the nature of the placenta as a membrane and by what means certain groups of substances are excluded by it, while others are allowed to pass; and to ascertain whether this reaction is comparable to others in the body or peculiar to the placenta alone. Finally it is desired to determine what rôle the placenta plays in the fetal metabolism, and to establish the nature of its relation to each type of substance that must be recognized as essential for the fetal nutrition.

MATERIALS AND METHODS

In this attempt to analyze the fluid interchange cats were used exclusively, and those in a late period of gestation were selected. The entire series consisted of animals that had passed the fortieth day of pregnancy. The youngest fetus in the series measured 78 mm., and the oldest 121 mm., crown rump diameter. The technique has been uniform throughout except in certain experiments designed expressly to test some specific point arising in the course of the work. All animals received balanced solutions of sodium ferrocyanide and iron ammonium citrate, the usual strength being 1½ per cent of each salt. The percentage strength was varied in some cases; these will be mentioned in the appropriate place. In all operations the solution was administered as an injection by means of a burette attached to a glass cannula introduced into the vein of the forearm. Intratracheal ether anesthesia was used in the majority of these experiments, and was found entirely satisfactory. It is well known that the cat maintains almost normal respiratory and circulatory functions under ether anesthesia. After establishing results which were entirely constant for ether, controls were decerebrated following Weed's (17) technique, and certain crucial experiments repeated. The results obtained were in all cases exactly the same as found in similar experiments under anesthesia. By this the possibility of any effect by the anesthetic on the placenta was eliminated, and this was essential since we know from Nicloux's (11) work that ether passes the placenta and it might in consequence render the placental membrane either more or less

permeable to the substances used in the experiment. The salts were weighed out and dissolved immediately before the experiment. They were not mixed until the cannula had been inserted, when they were poured together, and then directly into a burette and the injection begun. Both sodium ferrocyanide and iron ammonium citrate are eliminated very rapidly by the kidneys, so that some method had to be devised in order to keep the concentration of the salts constant in the blood stream. The method finally decided upon was the preliminary interruption of the renal excretion by ligation of the renal vessels. After this the desired amount of the experimental solution was run in slowly, and the animals kept undisturbed until sacrificed. In order to avoid the possibility of this procedure causing changes in blood pressure, experiments were performed to test the effect of administering these salts after ligation of renal vessels. Amounts more than double those usually given caused only a transient rise of pressure, so that the hydrostatic element may be ruled out. This method proved so satisfactory that it was used entirely, except for a few animals in which a continuous injection was maintained throughout the entire experiment of just enough solution of the salts to keep them present in the blood stream. Quantitative determinations were not made, but precipitation tests of Prussian blue in the maternal blood plasma were carried out in order to indicate the presence of both salts in approximately the same amounts, as in experiments done with the preliminary renal ligation. These experiments were considered necessary to exclude the possibility of the lack of excretion from the kidneys having an effect upon the placenta, but the results with the kidneys functioning and when ligated were the same. Cats were exposed to varying doses for periods of time ranging from ten minutes to ten hours, and were then killed. The uterus was opened so that the amniotic sac bulged out, the fluid carefully withdrawn with syringe and needle to avoid contamination, and then examined chemically. The fetuses were removed and their abdominal cavities opened; whenever the bladder was distended it was treated in precisely the same manner as the amniotic sac. Small blocks of fetal kidney and placenta were fixed directly in Bouin's fluid, the acetic acid in this mixture being sufficient to precipitate the Prussian blue. In the analysis of the fluids under examination, solutions of ferric chloride and ferric sulphate were used to determine the presence of sodium ferrocyanide, while a ferrocyanide was used to test for the ferric iron. It was found that there is sufficient iron present normally in fetal urine and amniotic fluid to yield a Prussian blue reaction with sodium

ferrocyanide and hydrochloric acid when allowed to stand; the test for ferric iron was therefore read immediately. The exact details of all tests will be given when the reports of the histological findings are made, and need not be considered further here. The results of the introduction of these salts into the blood stream of the living fetus are from a series of experiments, still quite incomplete, concerning the general methods of fetal excretion, and which will be reported later. Such materials as bear upon the present problem will be referred to here. In regard to the question of the validity of the tests given above the following experiment will indicate both the delicacy of the test and the certainty of finding our reagents in the fetal urine or amniotic fluid if they had reached the fetal circulation.

Cat P. P. 23. Intratracheal anesthesia. Midline abdominal incision with exposure of uterus.[1] One cubic centimeter of a solution containing 1 per cent each of sodium ferrocyanide and iron ammonium citrate was introduced into the umbilical vein of one of the fetuses, which was removed after two and a half minutes, and the bladder exposed. A drop of acid added to the fetal urine gave a brilliant blue color. Another fetus was exposed and the same procedure followed except that the time interval in this case was twenty minutes. The bladder was found to be contracted, test of the amniotic fluid yielded Prussian blue. The fetuses measured 80 mm.

It is quite evident that the fetal kidney will very quickly eliminate both salts, and that if the bladder has contracted after this elimination has begun the amniotic fluid will show their presence. We found, in many of our experiments on the fetus, that the bladders were empty. From this it seems probable that the increased flow of urine had stimulated them to contract. It is evident that the tests used were sufficiently delicate to indicate any exchange of these salts between mother and fetus even when relatively small doses were given to the mother intravenously.

EXPERIMENTAL RESULTS

The results of many experiments indicated that the placenta of the cat can be functionally divided into three parts in regard to its reaction to the salts here used. Duval (6), in studying the histology of the placenta, has described three layers in the labyrinth of the cat during later states of gestation: the maternal endothelium which remains intact and is included within the invading trophoblast of the ovum;

[1] The technique of these operations will be reported with the other details on embryonic excretion.

the ectodermal layer, which is syncitial in character; and the embryonic endothelium which lines the embryonic vessels even where they penetrate deeply into the syncytial ectodermal layer. The maternal endothelium is everywhere continuous, the cells being very prominent in contrast to the thin, delicate cells of the fetal capillaries. In every experiment of the entire series the reaction of the maternal endothelium was precisely the same. No variation in this reaction could be discovered in any part of the placenta, despite the fact that certain areas showed more blue than others on examination with the low power of the microscope. This was probably attributable to a variation in the blood supply due to mechanical causes. Everywhere on histological examination the characteristic blue granules could be seen both in the bodies of the endothelial cells and lying between them and the ectodermal layer. In exactly the same manner the fetal endothelium is easily permeable to both salts. This passage takes place very rapidly because in experiments of ten minutes duration, the blue granules are found in, and beyond, the endothelial cells. It is impossible to deny that the salts may pass between the endothelial cells as well as through them, although there are no granules precipitated in a manner which would indicate this, as they seem to be equally distributed throughout the entire extent of the cytoplasm. That they both pass through the maternal endothelium rapidly is evident and that they pass only through, and never between these cells is probable, but can not be stated as absolutely proved. This difficulty is obviously met with in any membrane, and has been commented upon by Weed (17) in regard to the arachnoidal villi; this will be referred to later. It is probable then that the maternal endothelium present in the placenta has the same degree of permeability in regard to these salts as endothelium elsewhere in the body. At least it can be asserted that they pass here in the same manner, that is, with the same histological distribution as was shown in the veins and capillaries of the omentum by Shipley and Cunningham (16). The maternal endothelium is easily permeable to both of these salts, the fetal endothelium is equally so, but the fetal ectoderm reacts in an entirely different manner. The behavior of the ectodermal layer permits the division of the experiments into two groups, relative both in terms of permeability and duration of time. In the first group are included experiments of duration varying from ten minutes to four hours and a half. In not one of these has any trace of either sodium ferrocyanide or iron ammonium citrate been demonstrated in amniotic fluid, fetal urine, or

tissue extract. And in every case the bladders of the fetuses were found on removal to be full of urine. In the second are included those from five to ten hours in duration, the latter being the maximum of the series. In all the experiments in this group traces of sodium ferrocyanide were found in the fetal urine or amniotic fluid, but in none of them could even the slightest trace of the iron ammonium citrate be demonstrated. The amount of ferrocyanide which had passed the placenta was in no case very large. In experiments of from five to six hours only a trace was found, this was increased somewhat in those of longer duration, the maximum being approximately that amount which would be excreted by a fetus in twenty minutes if ½ cc. of a 1 per cent solution had been injected intravenously. In marked contrast to the fetuses of the first group, the bladders were found in most cases to be contracted and entirely emptied of their urine. And in every one of these the ferrocyanide could be demonstrated in the amniotic fluid. This interesting reaction of the fetal bladder is illustrated in the following experiments:

Cat P. P. 25. 9:00 a.m. Intratracheal ether.

Each kidney delivered retroperitoneally, and the vessels ligated.

Cannula inserted into vein of left forearm of mother, connected to burette containing 50 cc. of solution of 1½ per cent each of sodium ferrocyanide and iron ammonium citrate.

9:20 a.m. Intravenous injection begun.
9:30 a.m. 5 cc. in.
9:40 a.m. 10 cc. in.
9:50 a.m. 15 cc. in.
10:00 a.m. 20 cc. in.
10:10 a.m. 25 cc. in. Injection stopped.

Animal in excellent condition. No change after injection began except increased salivation. The animal remained in excellent condition until sacrificed at 3 p.m. Total duration of experiment, five hours and forty minutes. The uterus was immediately removed and found to contain three fetuses in the left horn and two in the right. The uterine wall was incised over the caudal end of each fetus in such a manner that the amniotic sac bulged out freely; this was punctured with a needle and the fluid withdrawn. Each fetus was removed, all were noted to be alive. The abdominal walls were incised and the urine removed when present.

Right horn of uterus: Both fetuses had empty bladders, the amniotic fluids contained a trace of ferrocyanide, but no iron ammonium citrate. They measured 89 mm. and 92 mm.

Left horn of uterus: Fetus 1. Bladder full, but not distended. Urine negative for ferrocyanide and iron ammonium citrate. Measured 91 mm.

Fetus 2. Bladder full, and distended. Traces of ferrocyanide in urine, no iron ammonium citrate. Measured 90 mm.

Fetus 3. Bladder empty, trace of ferrocyanide in amniotic fluid; no iron ammonium citrate. Measured 94 mm.

Cat P. P. 28. 9:30 a.m. Intratracheal ether.

Each kidney delivered retroperitoneally and the vessels ligated.

Cannula inserted into vein of left forearm of mother, and connected to burette containing 50 cc. of solution of 2 per cent each sodium ferrocyanide and iron ammonium citrate.

9:50 a.m. Intravenous injection begun.
10:00 a.m. 5 cc. in.
10:10 a.m. 10 cc. in.
10:20 a.m. 15 cc. in.
10:30 a.m. 20 cc. in.
10:40 a.m. 25 cc. in. Injection stopped.

The only immediate effect of injection was increased salivation. Heart rate remained about same throughout.

6:20 p.m. 10 cc. of blood withdrawn from maternal heart, centrifuged immediately, a few drops of acid added, blue color developed.

6:20 p.m. Ether to death.

Total duration of experiment, eight hours and thirty-five minutes. The uterus was removed and found to contain four fetuses, two in each horn. The uterine wall of each fetus was incised carefully and the amniotic fluid removed and tested. The fetuses removed and their bladders examined. In three, the bladders were found to be entirely empty, and the amniotic fluid was positive for ferrocyanide and negative for iron ammonium citrate. In the fourth fetus the bladder contained a single drop of urine which gave a positive test for ferrocyanide with ferric chloride and the amniotic fluid reacted as in the other three fetuses. Tests for iron ammonium citrate were negative. The fetuses were all alive and measured 80, 82, 84 and 86 mm. respectively.

On histological examination of the placentae of animals subjected to experiments of this type, the reaction of the ectoderm is most remarkable. In the shortest experiments, of duration less than an hour, the Prussian blue is found throughout the maternal endothelium and in a fine blue line between this layer and the syncytial ectoderm. There are no granules to be seen anywhere within the ectoderm, but they are closely adherent to the outer border both of the syncytium and the inter-capillary giant cells. Indeed these present a most remarkable picture of large round cells everywhere surrounded by a fine blue border, but nowhere can any granules be seen within their cytoplasm. In experiments of longer duration, about two hours, blue granules are to be seen within the ectodermal layer; these have not penetrated far into the cytoplasm but tend to form lines relatively close to the maternal poles of the ectodermal nuclei. This arrangement is very irregular, frequently the granules collect in small masses instead of lines and filaments. In experiments of six, eight and ten

hours duration there is very little change in the histological findings. The blue granules are found deeper in the cytoplasm of the ectoderm, in some places they are scattered almost throughout the entire structure. None can be seen in the fetal endothelium, or between the endothelial cells and ectoderm. Also the inter-capillary giant cells have absorbed a small amount of the two salts so that a few fine blue granules are seen in the peripheral third of the cytoplasm, while the rest of the cell is entirely clear.

The principal advantage of the method used in these experiments is that, by the precipitation of Prussian blue in situ, it is possible to interrelate the results obtained by examination of fetal excretion with the histological location of the point at which these results must have been determined. These placentae are sufficienty interesting to merit a more detailed histological description than would be advisable here; this will be given later when comparative results on other species are available.

DISCUSSION

The principal facts demonstrated by these experiments are the differences in the permeability of the endothelial and ectodermal layers of the placenta, and the differences in the resistance offered to the two experimental salts by the fetal ectoderm. It is evident that the placenta may be considered as two protoplasmic membranes which are directly applied to each other in such a way that they appear anatomically as a single membrane separating the two bloods, but have in reality entirely different physiological properties.

In considering a given living membrane we have to deal with three phases of activity, the entrance of a substance into the membrane, its passage through the membrane, and its exit from the membrane. The factors involved in each of these may be entirely different, and those relating to one membrane may differ entirely from those relative to another. The simplest membrane in the organism is such a one as that described by Weed (17) in the arachnoidal villus where he found the exchange through the membrane to be effected by a simple hydrostatic filtration; osmotic pressure having little or no participation. That the passage of sodium ferrocyanide through the entire placenta is not a simple filtration dependent upon hydrostatic pressure alone, is evident from several experiments carried out expressly to determine this point. After animals had been exposed in the usual manner and to the usual dose of this salt, the blood pressure was raised to a max-

imal degree by the continued introduction of physiological saline under pressure of about 500 mm. of water. Despite this procedure no variation in the passage of this salt as previously determined in animals with normal blood pressure was noted. Simple filtration by hydrostatic pressure can therefore be considered as a negligible factor.

It is evident that all materials necessary for the nutrition of cells and all products of cell metabolism that either come from the blood stream or are eliminated by it, must pass through the capillary wall. Therefore the endothelial membrane must be permeable to water, salts and even to some colloids, since some of the substances included above are in that state. This remarkable degree of permeability shown by capillary endothelium has been explained in various ways. It has been suggested that the endothelial cells are capable of separating from each other and allowing large molecules, as well as small, to pass through by simple filtration. Others have thought that the intercellular cement substance is physiologically inert and contains large pores through which diffusion of colloids may take place. The other possibility is, that the endothelial cells are constructed in such a way that their cytoplasm is permeable to large molecules. The nature of the mechanism by which permeability is regulated still remains unsettled; Bayliss (4) after reviewing many observations at variance with each other concludes that:

> Different views are held as to that property of a membrane which makes it permeable to some solutes and impermeable to others. Reasons are given in the text for accepting, with some modifications, the original sieve theory of Traube, according to which the passage of a solute through a particular membrane, depends on the size of the pores in the membrane in relation to the molecular, or particulate, dimensions of the solute. The hydration of solutes must be taken into account. In a few cases, the question of solubility in the substance of the membrane appears to play a part.

That capillary endothelium is permeable to both iron ammonium citrate and sodium ferrocyanide has been shown in the case of the omentum by Shipley and Cunningham (16). The forces involved in the passage of these salts through the maternal endothelium of the placenta are probably no different from those operative in the case of any other capillary wall. That osmosis and diffusion are the most important is assured, but that other factors are involved is shown by the observations that substances can be absorbed from the peritoneal cavity against osmotic pressure and concentration gradient, and likewise substances can be secreted by the kidney under similar circum-

stance. While these factors are not yet entirely understood they are explained in terms of negative osmose, which in turn is explained in terms of electro-endosmose, or the pull of a charged particle through a membrane pore by an opposite charge on the membrane (Bartell and Hocker (3)). The experiments in this series do not assist in analysing the factors pertaining to the permeability of the maternal endothelium, and therefore of endothelium in general, except that they show conclusively the passage of these salts through the cytoplasm of the cells.

We have seen that both sodium ferrocyanide and iron ammonium citrate penetrate the ectodermal layer in about two hours, while the majority of cells in the mammalian organism appear to be semi-permeable as regards these salts, even when exposed to them for much greater periods of time. So that the limiting membrane of the fetal ectoderm must differ in its normal permeability from the limiting membrane of cells in general. The differences in structure or composition between membranes permeable to certain crystalloids and others semi-permeable to them, are as yet unknown. Whether other factors than osmosis and diffusion are concerned in the penetration of the ectodermal membrane can not now be stated. When we consider the passage of these substances through the cytoplasm and the exit of one while the other is retained, other factors must be considered. After about five hours the sodium ferrocyanide passes through the cell membrane adjacent to the fetal capillaries and can be detected very quickly in the fetal urine. At this time tests fail to reveal any citrate as having passed through, nor do experiments of several hours longer duration yield different results. It is evident, therefore, if these tests are sufficiently accurate, that no citrate has been able to pass out of the fetal ectoderm despite the fact that it has unquestionably entered it. The rate at which sodium ferrocyanide passes through the placenta is extremely slow in comparison with its passage through other living membranes which have been studied. While this does not prove, it certainly suggests that other factors than osmotic pressure and diffusion are involved. It is possible however, to conceive that the ectoderm is so difficultly permeable to sodium ferrocyanide that we are actually measuring the normal time element of its diffusion, in other words, the ratio of osmotic pressure to the resistance of membrane. The determination of the osmotic pressure of the maternal and fetal bloods would be of great assistance in indicating how much salt exchange depends on this factor. Such determinations have not been

done. It is evident that if an osmotic balance is maintained this exchange would be easier than if the presence of metabolic products in the fetus should have raised the pressure on that side; but it is possible to conceive that even if the osmotic pressure in the fetal blood is equal to, or lower than in the maternal blood, the retardation of the passage of sodium ferrocyanide might be caused by an unstable combination with intracellular compounds; on the other hand, if the fetal pressure be proved to be higher, some activity comparable to that of secretion must be postulated.

We can conclude much more surely that other factors than osmosis and diffusion are operative in regard to the peculiar action of the ectodermal membrane toward the iron ammonium citrate, because both the ferrocyanide and citrate are known to diffuse through many different membranes at approximately equal rates. Of course it must be admitted that we have not excluded the possibility of the citrate passing through the ectodermal layer if exposed for a longer period of time. In this case we would be forced to conclude either that some intracellular activity had delayed the citrate longer than the ferrocyanide, or that the fetal surface of the ectoderm was much more easily permeable to ferrocyanide than to the citrate, and consequently different, not only from many other living membranes, but also from the maternal side of the ectoderm. On the other hand, if longer experiments substantiate those of six, eight and ten hours duration, we are forced to the conclusion of some even more radical change taking place within the fetal ectodermal layer. Two possibilities may be mentioned, although it is evident that both are entirely without experimental evidence. First, it is conceivable that some substance present in the cell prevents the further passage of the citrate by a specific adsorption. Second, as many organic acids and salts of organic acids are changed and broken down in the metabolism of the animal body, it is therefore quite possible that the ectodermal syncytium is capable of breaking down iron ammonium citrate into substances no longer detectable by our tests, which were after all only capable of demonstrating the presence of ferric iron. It should certainly prove feasible to devise methods for determining whether this salt, and perhaps many other salts of organic acids, can be decomposed in the fetal ectoderm. We have considered so far, in endeavoring to explain the results of our experiments, the effects that the tissues involved may have had upon the salts introduced; but it is necessary to consider also what effect the salts may have had upon the tissues. While the death of the cell after exposure

to these salts can be easily determined by the brilliant blue staining of the nucleus, minor injuries do not produce any change which can be recognized in histological preparations. In general it is known that permeability of cells is increased by injury; if then the ectoderm has been changed by these salts we must conclude that it is normally semi-permeable to them. But it is extremely difficult to understand how one of these salts can be retained and the other pass out into the fetal blood, if they can not penetrate the ectoderm until they have injured it in some degree. Admitting that it is injured, the results which have been obtained can only be explained in one of two ways: either the injury is insufficient to destroy the activity which prevents the passage of the citrate, or else this function is a chemical reaction developed as the result of the injury. These explanations are both rather improbable, so that it seems more likely that the degree of injury is slight. Whether or not longer exposure to these salts, and particularly to the citrate, would depress the cell activities enough to allow the citrate to pass through is entirely problematical and need only be stated here as a possibility. Finally, it is worth while to consider what bearing these experiments have upon the theory advanced by Bremer and referred to before. According to his theory excretory products of fetal metabolism in the cat do not pass through the placenta, but are excreted by the fetal pronephros, Wolffian body or kidney, as the case may be. Since the fetal as well as the adult kidney excrete both of these salts very rapidly, i.e., within two or three minutes, and since it requires five hours for one of them to pass the placenta, it seems strong evidence that his interpretation of the absence of placental excretion in the cat is correct. Although still quite incomplete, the work on the excretion of the embryo, referred to above, also substantiates Bremer's view as far as the cat is concerned.

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THE PRODUCTION OF INTRACELLULAR ACIDITY BY NEUTRAL AND ALKALINE SOLUTIONS CONTAINING CARBON DIOXIDE

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It has recently been pointed out by the author (1) that the apparently contradictory views as to the mode of action of CO_2 on the mammalian respiratory center expressed on the one hand by Winterstein (2), Hasselbalch and Lundsgaard (3), (4) and others, and on the other by Laqueur and Verzár (5), Hooker, Wilson and Connet (6) and Scott (7) are not necessarily in conflict. Carbonic acid may conceivably, like any other acid, act primarily through its hydrogen ions, as the first group of workers have held, and yet differ so greatly from other acids in certain peculiarities connected with its powers of penetrating living cells as to have practically as specific an action as that postulated by the second group.

One of the most interesting of these peculiarities is the relatively small importance of the hydrogen ion concentration of a solution containing carbon dioxide in determining the degree of intracellular acidity produced in a cell exposed to it. The author has already called attention to the fact that a neutral or even a slightly alkaline CO_2-bicarbonate mixture is practically as toxic to tadpoles as a pure CO_2 solution of the same concentration, and that such a solution has a sour taste. He has interpreted these results as being due to the fact that the H_2CO_3, whose dissociation is held in check by the bicarbonate, can freely enter cells (perhaps as CO_2), while the bicarbonate cannot, the carbonic acid dissociating when within the cells to the extent permitted by the new conditions of equilibrium prevailing there, which may easily result in a hydrogen ion concentration higher than that of the surrounding medium.

While the observations already recorded have probably been correctly interpreted, it has seemed desirable to supplement them with a case where the rise in intracellular acidity under the conditions in ques-

tion is actually visible to the eye. Such a case might be furnished by a cell containing a sufficiently sensitive natural indicator whose color changes would give visible evidence of any increase in the hydrogen ion concentration within the cell. After a rather lengthy search, material of this character has been found in the colored flowers of *Symphytum peregrinum*, a cultivated plant belonging to the family *Boraginaceae*. These flowers, like those of many other members of the family, are pink in the bud, later becoming blue; the former color is associated with a higher, the latter with a lower hydrogen ion concentration. The exact pH of the turning point has not been determined, but is within the range of carbonic acid solutions.

The use of colored flowers to study cell penetration by acids, though Haas (8) obtained good results with this method, has not been very much favored in the past because of the fact that such cells are generally cuticularized and are not readily wetted by aqueous solutions. In the present case, however, it was found that if the end of the tubular corolla were snipped off with a pair of scissors and the whole flower were then slipped over the tapering end of a glass rod which could be dropped into a test-tube containing the solution to be studied, the change in color appeared quickly and regularly and could easily be followed, especially along the "ribs" correspondng to the attachment of the stamens, and at and above the depressions which are found at the points of attachment of the "corolla scales." To observe the change in color, a hand lens was used, though even with the naked eye the results were easily visible. For example, the difference between a flower exposed to a saturated solution of CO_2 and one kept in distilled water was usually apparent in five minutes at a distance of five or six feet. The change in color with weak solutions of acids always began on the "ribs" and above the depressions mentioned, and gradually spread from them to other parts of the flower.

As a considerable number of similar experiments with this material all gave essentially the same results, it will be sufficient to describe a typical one. Four flowers from the same plant, as nearly alike in color as possible, were selected. The first was exposed to a saturated solution of CO_2 in distilled water (pH approximately 3.8); the second to pure distilled water of pH between 5.0 and 6.0 (the slight acidity being due to CO_2 absorbed from the air); the third to an M/2 solution of $NaHCO_3$ saturated with CO_2 at atmospheric pressure and having a pH of *ca.* 7.4, and the fourth to an M/2 solution of $NaHCO_3$

considerably more alkaline than pH 8.0. The course of the experiment may be indicated most clearly in tabular form as shown in table 1.

It will be observed that the increase in acidity of the cells was not proportional to the pH of the external medium, since those exposed to

TABLE 1

Color changes in flowers of Symphytum exposed to various solutions

TIME	(1) DISTILLED WATER + CO_2	(2) DISTILLED WATER	(3) M/2 $NaHCO_3$ + CO_2	(4) M/2 $NaHCO_3$
11:20*	Blue	Blue	Blue	Blue
11:21	Pink color appearing on lower "ribs" and above depressions	No change	Pink color appearing on lower "ribs" and above depressions	No change
11:23	Upper "ribs" becoming pink	No change	Upper "ribs" becoming pink	No change
11:25	Pink spreading laterally from lower "ribs"	No change	Pink spreading laterally from lower "ribs"	No change
11:27	Lower portion of flower mostly violet with pink "ribs"	No change	Lower portion of flower mostly violet with pink "ribs"	No change
11:31	Upper portion of flower shows considerable pink and violet outside of "ribs"	No change	Upper portion of flower shows less violet than (1) but "ribs" are, if anything, pinker	No change
11:38	About the same	No change	About the same	No change
11:45	Same	No change	Same	Becoming slightly bluer than (2)
1:05	Same	No change	Slightly bluer than before but still pinker than (2)	Beginning to turn greenish
2:30	Same	No change	About the same as (2)	Decidedly greenish

* Started.

the slightly akaline CO_2-bicarbonate mixture became distinctly acid, while those in the distilled water with a hydrogen ion concentration perhaps one hundred times as great did not. There was also comparatively little difference (up to the time when the bicarbonate finally

began to penetrate) between the two solutions saturated with CO_2, though the concentration of hydrogen ions in the one was approximately four thousand times that in the other. Evidently, therefore, the neutrality or slight alkalinity of a solution does not rule out the possibility of decided effects of hydrogen ions where CO_2 is one of the substances concerned, and the stimulation of the mammalian respiratory center in Scott's (7) experiments by blood of pH 7.6 would not necessarily be in conflict with the orthodox view of the rôle of hydrogen ions in this process.

It may perhaps be of interest to show that the effects of CO_2 on living cells here described may be imitated by a simple model whose construction depends on the fact that CO_2 is freely soluble in xylene (as well as in other lipoid solvents and lipoids) while $NaHCO_3$ is not. The "cell" is made as follows. A small "shell vial," 10 by 35 mm., is filled to within 4 mm. of the top with a solution of phenolsulphonephthalein made very slightly alkaline by the addition of a trace of $NaHCO_3$. It is next filled level full with xylene (from which the CO_2, if necessary, has been removed by allowing it to stand in contact with an aqueous $Ba(OH)_2$ solution, and the escape of the xylene is then prevented by the following simple expedient. A test tube is dipped into a fairly thick celloidin solution and the film which closes its mouth on removal is immediately pressed over the opening of the vial to which it adheres as a thin transparent covering, freely permeable to salts, acids, etc., but preventing the loss of the xylene when the "cell" is immersed in water. The vials as thus prepared are kept until needed in an upright position in water. In some cases cottonseed oil was used in place of xylene with essentially similar results, though it is less convenient to work with, and results are obtained more slowly with it than with xylene.

A typical experiment with "artificial cells" was the following. The solutions used were the same as those studied with the *Symphytum* flowers, namely, distilled water saturated with CO_2, distilled water, M/2 $NaHCO_3$ saturated with CO_2, and M/2 $NaHCO_3$. With the distilled water and $NaHCO_3$, there was no change in the color of the indicator; with the two solutions containing CO_2, though, as before, the actual hydrogen ion concentration of the one was perhaps four thousand times as great as that of the other, there was an approximately equal rate of change in color of the red indicator solution to a bright yellow. In both cases, the change began to be visible within two

minutes and was complete in about an hour and a half. The line of demarcation between the red and the yellow portions of the solution was very sharp, especially at first.

The result of one alkaline solution turning acid when placed in another alkaline solution is a rather striking one. In this case it evidently depends on the relative solubilities of CO_2 (and perhaps H_2CO_3), on the one hand, and of $NaHCO_3$ on the other, in lipoids and lipoid solvents. In living cells there is frequently a close correlation between the lipoid solubility of substances and their powers of penetration. Without at all postulating a lipoid membrane in Overton's original sense, it is nevertheless possible that the relative solubilities of the substances concerned in the lipoid and aqueous phases encountered in the structure of a typical cell may have much to do with the physiological behavior of CO_2-bicarbonate mixtures.

In connection with the points already mentioned, it is of interest to compare the penetrating powers of CO_2 with those of other acids, particularly those which, from the results of previous workers, appear to enter living cells with the greatest readiness. Such a comparison was in progress at the time when a severe storm put a temporary end to the available supply of *Symphytum* flowers. Reserving, therefore, for a subsequent paper the details of the work already done and certain points requiring further elucidation, it may be said briefly that of all of the acids studied (carbonic, benzoic, salicylic, valeric, butyric, acetic, sulphuric and hydrochloric), carbonic is by far the most effective when pure solutions *of equal pH* are compared, in causing a visible change in intracellular acidity. For example, with a solution saturated with CO_2 at ordinary temperatures, a visible change in color usually begins in one or two minutes, while with the acids next in the order of their effectiveness (benzoic and valeric) fifteen to thirty minutes are required, with butyric, acetic and salicylic acids following in the order named. The mineral acids are only very slightly effective.

These results, as far as the acids other than carbonic (which apparently has not yet been studied in this manner) are concerned, agree fairly well with the findings of Haas (8) and of Collett (9) and also with the observation of the author (1) that tadpoles are fatally injured in saturated CO_2 solutions in two or three minutes, while in solutions of other acids of the same pH, from one to several hours are required to produce the same result.

If carbonic acid be compared with other acids of the same normality instead of the same pH, it appears far down the list, as would be ex-

pected, not because it does not enter cells readily—for there is much evidence that it does—but because, on account of its weakness (i.e., its low degree of dissociation), it is necessary for so much more of it to enter the cell before a change in the color of the indicator can occur than in the case of the more strongly dissociated acids.

One further point may be noted. If a solution, e.g., N/10, of NaOH have various acids added to it (in as concentrated a form as possible to avoid dilution) until the point of neutrality, as shown by phenolsulphonephthalein is reached, there should at this point, in the case of fairly strong acids, be practically no free acid present, while in the case of a weak acid like H_2CO_3, there should be a considerable amount. This difference may easily be made visible in the case of carbonic and other lipoid-soluble acids, such as benzoic, salicylic and butyric, by using the "artificial cells" already described. In one such experiment where the solutions were all neutral and N/10 with respect to total base, the indicator solution within the vial began to change color in three minutes with carbonic acid, while with butyric, benzoic and salicylic acids (all of which are highly effective with the "artificial cells" in the free form on account of their solubility in xylene) no change had occurred in three hours. Had the water used been so free from CO_2 as to make possible the use of an indicator solution with a lower buffer value, the effect of the smaller amounts of the other acids could probably have been detected as well, but as the experiment was not intended to be accurately quantitative, but merely to show that a very considerable difference exists between carbonic and the other acids, it was not considered necessary to take this precaution.

As the final conclusion to be drawn from the various experiments described in this paper, which have been made partly on living plant cells and partly on a simple "artificial cell," it may be stated that the physiological behavior of CO_2 probably depends to a considerable extent on two of its chemical and physical peculiarities: *a*, the weakness of H_2CO_3 as an acid, permitting the existence of a relatively large amount of the free but undissociated acid (as well as dissolved CO_2) in the equilibrium that exists at neutrality or slight alkalinity; and *b*, the readiness with which the undissociated acid, or its anhydride, CO_2, enters living cells, perhaps in virtue of its lipoid solubility. The combination of these two peculiarities is responsible for certain of the remarkable, and in some respects, unique, physiological properties of carbon dioxide.

SUMMARY

1. It has been found that the flowers of *Symphytum peregrinum* contain a natural indicator sensitive to carbonic acid, which may be used to study cell penetration by CO_2.

2. Using this material, it appears that a condition of intracellular acidity can be produced by a slightly alkaline solution of CO_2 in M/2 $NaHCO_3$ almost as effectively as by a solution of CO_2 in distilled water, though the hydrogen ion concentration of the latter solution is approximately four thousand times as great as that of the former.

3. A similar result may be obtained by the use of an "artificial cell" whose construction is described.

4. From pure aqueous solutions of acids of the same pH, carbonic acid changes the color of *Symphytum* flowers more quickly than do any of the other acids studied.

5. The ability of neutral and slightly alkaline solutions containing CO_2 to produce intracellular acidity is probably due to at least two factors: *a*, the weakness of H_2CO_3 as an acid and *b*, the lipoid solubility of CO_2 or H_2CO_3 or both, and the lack of such solubility in the case of bicarbonates.

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THE EFFECT OF SALT INGESTION ON CEREBRO-SPINAL FLUID PRESSURE AND BRAIN VOLUME

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Recently Weed and McKibben (1), (2) demonstrated the important physiological fact that it is possible to reduce the cerebro-spinal fluid pressure and diminish the bulk of the brain by the simple procedure of injecting a hypertonic solution into the blood stream. Dr. Harvey Cushing, appreciating the clinical possibilities of this discovery, was led to suggest the present study on the following speculations:

It is common knowledge that people who suffer from headaches suggesting "tension headaches" may get relief by a thorough intestinal evacuation, particularly when this is accomplished by salines. This has led to the view that constipation in itself is provocative of such discomforts. It would be of profit to repeat the observations of Weed and McKibben and then attempt to accomplish the same results by giving the hypertonic saline by mouth instead of intravenously. It seems possible that the withdrawal of fluid from the blood may increase its salt content sufficiently to cause the same result but without actually introducing salt into the blood stream. The fact established by Weed and McKibben might be adaptable to certain cranial operations, making them easier by lowering tension and diminishing brain volume.

INTRODUCTORY

In this laboratory some years ago, L. H. Weed began a series of experiments in an effort to answer certain questions relating to the cerebro-spinal fluid circulation which had been suggested by Doctor Cushing (3). His studies related largely to the circulation of the fluid and to the methods of its escape from the cerebral chamber (4). It was demonstrated that the normal escapes for fluid were by way of the arachnoid villi directly into the large dural sinuses and along the perineural spaces about the cranial and spinal nerves. He subsequently showed how the arachnoid spaces and dural sinuses developed (5).

On the side of pure physiology we are not so secure. It has recently been claimed that a solution of the primary problem of fluid produc-

tion has not been accomplished. There is much presumptive evidence of an experimental and clinical nature that the choroid plexus behaves as a secreting gland in this connection. Such has been the generally accepted belief. Later students of the subject, and in particular Becht (6) and Becht and Matill (7), have objected to some of these generally accepted broad facts. They have recently published the results of a very critical reinvestigation of the subject and conclude that there is no indisputable evidence that the fluid is a secretory product of the choroid. Dixon and Halliburton (8), 1913, published an elaborate investigation of the action of certain drugs and tissue extracts on the "secretion." Their results are somewhat discounted by the work of Becht and Matill who point out that the results obtained may have been due to intracranial vascular (pressure and volume) changes caused by the injected substances and recorded as fluid secretion changes through the inadequacy of the methods employed. These authors applied the same criticism to that part of the work of Cushing and Weed (9) in which they claimed to demonstrate an increased secretion of fluid following the administration of hypophysis extract. The criticism does not seem warranted since in some of their experiments Cushing and Weed measured the increased flow of fluid from a catheter within the ventricle. The catheter was sealed off from the subarachnoid space by its contact with the walls of the aqueduct. Under such circumstances the changes in capacity of the subarachnoid space, incident to vascular changes, could not impart a significant alteration to the flow from the ventricular catheter.

Aside from the primary broad facts of anatomy, secretion and absorption channels, the work of Weed and McKibben brings out the most definite and significant fact established in regard to the detailed physiology and pharmacology of the cerebro-spinal fluid. The demonstration that intravenous injections of hypertonic solutions reduce cerebro-spinal fluid pressure and brain bulk offers a new point of departure into the problems of the physiology and pathology of the cerebro-spinal fluid.

A brief summary of the work of Weed and McKibben (loc. cit.) is essential to a presentation of the present study. In experiments on cats it was shown that hypertonic solutions of electrolytes injected into the venous circulation caused a marked fall of the cerebro-spinal fluid pressure. Hypertonic solutions of other crystalloids (glucose) caused a similar fall of pressure though not so great in extent. Saturated solutions of sodium chloride, sodium bicarbonate and sodium sulphate

were employed. Conversely, it was found that distilled water (a hypotonic solution) had the opposite effect and caused a rise of pressure. It was later observed that the hypertonic solutions caused the brain to diminish in bulk while the hypotonic solution had the opposite effect, the brain increased in bulk. It was suggested that these changes were mediated through changes in the osmotic value of the blood.

EXPERIMENTAL

In experiments of this sort the methods employed are important to a proper interpretation of results. For this reason the method employed is set forth in considerable detail.

Animals. Most of the experiments were made on cats. The animals were taken from stock, usually without any preparation. In a few instances castor oil was given twenty-four hours before experiment. Some of the animals were allowed no food or water for this period. These measures did not appear to affect markedly the results and were given up. The animals were securely fixed in the prone position, the neck flexed and the head held tightly in place. For the recording of accurate pressure changes the fixation and position of the animal are most important for slight movements greatly affect the pressure.

Anesthetics. We have used the following anesthetics: Ether *a*, by cone and *b*, by the intratracheal method; *c*, morphine-atropine-urethane; *d*, luminal and *e*, morphine-chloretone. In our experience morphine-atropine-urethane is the most satisfactory anesthetic for the experiments on cats. The anesthesia requires no attention, it is uniform throughout the experiment and appears to have little effect on the fluid pressure. Chloretone does almost as well. With ether anesthesia the animals lose heat so rapidly that even with them placed on electric pads and under the warmth of several electric bulbs we have found it impossible to keep them in good condition for long periods of observation (8 to 10 hours). Changes in the depth of ether anesthesia markedly affect the intracranial fluid-blood pressure relationship causing changes quite apart from the purpose of experiment. The animals eventually fail when morphine-atropine-urethane is used, but in our experience it gives the most satisfactory period for observation.

Manometer and connections. The fluid pressures were read from a U-tube manometer connected to a needle in the subarchnoid space. The method of establishing the manometer connection is of importance. The whole system was filled with normal saline, the column of

fluid being set at 100 mm., somewhat below the initial pressure usually to be anticipated. A wire obturator extended through a water-tight puncture in the tubing on into the needle. With the apparatus so arranged the puncture through the occipito-atlantoid ligament was made. The obturator was withdrawn from the needle but its end was still left in the tubing to occlude the puncture through which it was inserted. By this method we were able to avoid the great loss of fluid and artificial fall of pressure incident to beginning with an empty system into which the fluid must rise to fill the manometer. The bore of the manometer was such that 100 mm. pressure change required a displacement of 0.26 cc. of fluid.

The importance of the displaced fluid in experiments of this sort has been emphasized. It will vary directly with the bore of the manometer. The possible bearing of this fact on the results was realized and so a number of control experiments with a larger bore manometer were conducted in which 100 mm. pressure change required a displacement of 1.06 cc. of fluid.

Arterial and venous pressures. These were recorded in some of the first experiments. It soon became apparent that they were not essential to an interpretation of the enormous fluid pressure changes that occurred and so their recording was given up.

Routes of administration: Intravenous doses were given from a buret through a cannula in the femoral vein. It was possible to control accurately the rate of flow. *Gastro-intestinal doses* were given through a stomach tube. *Duodenal doses* were given by a syringe and needle into a loop of duodenum exposed through a small mid-line incision. *Colonic doses* were given by a rectal tube held in place by a purse string suture in the anus.

Normal pressures. We wish first to record the values we have obtained for normal pressures. In addition to the present series Weed and McKibben (loc. cit.) and Becht (loc. cit.) furnish the most comprehensive data on the subject. In a series of one hundred experiments on cats under the experimental conditions described, the average of normal pressures of the cerebro-spinal fluid was 133 mm. normal saline. This is 14 mm. higher than the value of 119 mm. given by Weed and McKibben and 21 mm. higher than the value of 112 mm. given by Becht. The former authors cited used an empty manometer into which the fluid had to rise. It is not clear whether or not Becht began with a filled system in the experiments on which this figure is based. At any rate, this factor in our experiments probably, in part, explains

the higher value of our normal pressures. When an appreciable amount of fluid has been displaced it takes a considerable period of time for a new equilibrium to be established. Under these circumstances readings taken early in an experiment only represent a point in the transition toward equilibrium. The average normal with ether anesthesia was 127; with urethane 155 and with chloretone 127. The higher value of our normal readings may be connected with the use of urethane. The highest initial pressure we recorded was 255 mm. The lowest positive pressure was 65 mm. In three animals the pressure at the beginning of experiment was negative, viz., −10 mm., −20 mm., −25 mm. These values existed after the 100 mm. column of fluid in the manometer (0.26 cc.) had run into the subarachnoid space. The experimental details were carefully checked up in these cases so that we are sure the figures represent true values.

INTRAVENOUS DOSES

A number of experiments in which the solutions were given intravenously were carried out. They abundantly confirmed the work of Weed and McKibben (loc. cit.). In addition they furnished a basis for comparison between intravenous and gastro-intestinal doses of the solutions.

Ringer's solution regularly caused a marked though unsustained rise of the cerebro-spinal fluid pressure. For example an intravenous dose of 50 cc. was followed by a rise of the cerebro-spinal fluid pressure of 100 mm. Within an hour the pressure had fallen to approximately a normal value again. On the other hand, the same amount of water given intravenously caused a marked and sustained rise of the cerebro-spinal fluid pressure (100 mm.) which, an hour after the injection, was still at its height. These changes were not accompanied by significant changes in the blood pressure. Figure 1 illustrates the effect on cerebro-spinal fluid pressure and blood pressure of an intravenous injection of 25 cc. 30 per cent sodium chloride solution. The cerebro-spinal fluid pressure promptly fell from 175 mm. to −78 mm. The fall is sustained, for two hours and fifteen minutes after injection the pressure remains 197 mm. below its level at the beginning of experiment.

These experiments are typical of our results with intravenous injections. The changes are greater than those recorded by Weed and McKibben but qualitatively they are identical with their experiments. In longer continued experiments we have found the lowered cerebro-

spinal fluid pressure persisting for long periods. In one experiment, for example (10 cc. 30 per cent NaCl intravenously), it remained 110 mm. below its initial level five hours and twenty minutes after injection. In this experiment a fall of 238 mm. took place in fifty-four minutes or at the rate of approximately 4.4 mm. per minute. The return toward normal occurred more slowly, at the rate of approximately 0.7 mm. per minute.

Unless the rate of injection of the hypertonic sodium chloride is carefully controlled the cardiac and respiratory changes may prove fatal. Death from these causes occurred in several experiments.

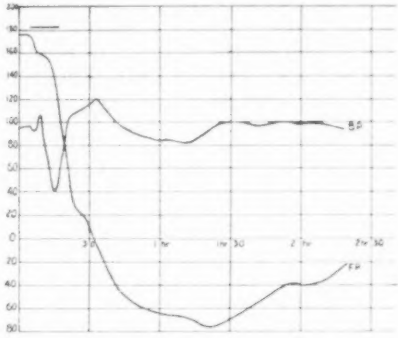

Fig. 1. Intravenous injection of 25 cc. 30 per cent sodium chloride solution. Dog weight 6.0 kgm. Ether by cone. Cerebro-spinal fluid pressure (mm. saline) and blood pressure (mm. Hg.) curves. During the injection there was marked cardiac and respiratory disturbance—the respirations became rapid and shallow, almost ceasing, the heart rate rose and the beats became feeble. Note the 70 mm. fall of arterial pressure during this period. A prompt recovery occurred with return to a higher value than previously. An abrupt fall, 253 mm. of cerebro-spinal fluid pressure occurred. Two hours and fifteen minutes after injection this pressure remains at −22 mm. or 197 mm. below the level at the beginning of experiment. The change is independent of blood pressure.

GASTRO-INTESTINAL DOSES

General effect. By a single experiment our main anticipation in regard to salt ingestion was shown to be well warranted. To an anesthetized dog a massive dose (180 cc.) of 30 per cent sodium chloride was given by stomach tube. The cerebro-spinal fluid pressure promptly fell from 150 mm. (normal saline) to zero. This was accompanied by a rising arterial pressure during the whole experiment. This is the essential fact. The subsidiary facts—size of dose, response to different substances, vascular changes, duration of the effect and so on—will be presented in the following experiments.

Size of dose. Figure 2 illustrates the effect on cerebro-spinal fluid pressure and blood pressure of injecting 35 cc. 10 per cent sodium chloride solution into the rectum. Five minutes after the injection

was begun the cerebro-spinal fluid pressure began to fall. In one hour and ten minutes it had fallen from 112 mm. to −44 mm., a drop of 156 mm. This was at the rate of 2.2 mm. per minute. The change is quite independent of the blood pressure which, as may be seen, remains quite constant. The change here is not so extensive nor rapid as in the case of intravenous doses—compare figure 1 with figure 2.

That the extent and rate of change obtained in this experiment are not the maximal ones to be obtained by intestinal doses was soon apparent. Figure 3 illustrates this fact. In this experiment 50 cc. of

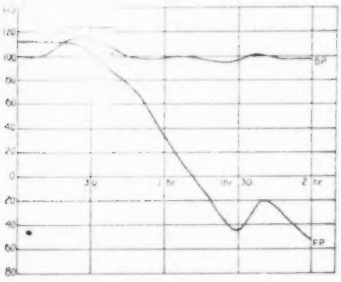

Fig. 2. Thirty-five cubic centimeters 10 per cent sodium chloride solution by rectal tube. Cat weight 2.5 kgm. Intratracheal ether. Cerebro-spinal fluid pressure (mm. saline) and blood pressure (mm. Hg.). Soon after the injection is begun the cerebro-spinal fluid pressure begins to fall—156 mm. in 1 hour and 10 minutes. Rate 2.2 mm. per minute. The arterial pressure rises a little during the injection but shows no significant change.

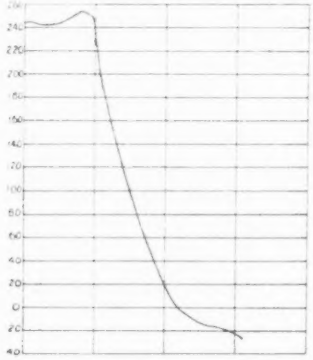

Fig. 3. Fifty cubic centimeters 30 per cent sodium chloride by rectal tube. Cat weight 3.2 kgm. Intratracheal ether. A fall of 270 mm. occurred at the rate of 4.2 mm. per minute. The curve closely resembles that obtained with intravenous doses, an example of which is shown in figure 1.

30 per cent sodium chloride solution were put into the rectum. The fall of pressure and the rate at which it occurred were here equal to intravenous doses, a fall of 270 mm. at the rate of 4.2 mm. per minute, compare figure 1 with figure 3.

Changes of this extent are maximal and larger doses add nothing to the effect. Much smaller doses, 20, 10, 5 cc. of 30 per cent salt solution still cause very marked falls of pressure. An injection of 5 cc. of 30 per cent sodium chloride solution into the duodenum caused a fall

of 104 mm. in the cerebro-spinal fluid pressure. Comparing this with the injection of a similar amount of the same solution intravenously, little difference between the effects produced is found. The intravenous dose causes a slightly more rapid fall.

Route of administration. As between rectal, duodenal and gastric doses little difference in the effect is to be noted when large doses are employed. Smaller doses, however, are most efficient in the duodenum.

Concentration of solution. Solutions of much lower concentrations than those mentioned so far are capable of producing changes of the same sort. Here, however, the rate of change is much slower and the fall of pressure is decidedly less in extent. In a number of experiments the average maximal effect to be obtained with large doses (40, 50, 150 cc.) of 2 per cent sodium chloride per duodenum was a fall of 97 mm. pressure at the average rate of 2.5 mm. per minute. Figure 4

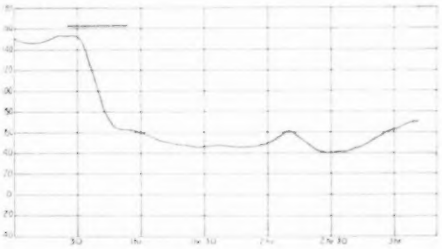

Fig. 4. Injection of 150 cc. 2 per cent sodium chloride solution, containing 2.7 per cent magnesium sulphate into the duodenum. Cat weight 3 kgm. Urethane anesthesia. Curve of the cerebro-spinal fluid pressure changes (mm. saline).

represents an experiment of this sort. The average maximal effect obtained with the large doses (20 to 35 cc.) of 30 per cent sodium chloride on the other hand was a fall of 258 mm. pressure at the average rate of 3 mm. per minute.

The large volume of solution in the case of the doses of lower concentration probably brought the solution into contact with a larger area of intestinal mucosa than was the case with the smaller volume of solution when 30 per cent salt was used. Were it not for this factor, the lower concentrations would have very likely affected the change at a still slower rate.

Comparison with intravenous doses. These large doses of concentrated salt solution in the gastro-intestinal tract cause maximal effects

as regards the extent of the fall of pressure. Comparing the results of such experiments with those in which the intravenous route was used the extent of fall in the two cases is approximately equal. The intravenous doses produce the effect at a slightly more rapid rate.

Duration of effect. Some of the experiments were continued for long periods. It was found that the lowered pressures endured for a considerable period. The curves are too long for reproduction but the following tabulation of four experiments (table 1) illustrates the point.

TABLE 1

Cerebro-spinal fluid pressures at hour intervals following salt ingestion

	INITIAL	HOUR INTERVALS								
		1	2	3	4	5	6	7	8	9
10 cc. 30 per cent NaCl	160	−78	−35	+4	+32	+45				
20 cc. 30 per cent NaCl	240	−40	−50	0	+60	+110	+140	+130	+150	+145
20 cc. 30 per cent NaCl	120	−70	−85	−40	−2	+20	+38	+55		
10 cc. 30 per cent NaCl	230	0	+40	+84	+138	+182				

TABLE 2

Cerebro-spinal fluid pressures at long intervals after salt ingestion

	PRESSURE WHEN PUNCTURED	NORMAL	DIFFERENCE
20 cc. 30 per cent NaCl 48 hours before puncture 20 cc. 30 per cent NaCl 24 hours before puncture	100	133	33
15 cc. 30 per cent NaCl 22 hours before puncture 15 cc. 30 per cent NaCl 18 hours before puncture	75	133	58
35 cc. 30 per cent NaCl 17 hours before puncture	55	133	78
35 cc. 30 per cent NaCl 22 hours before puncture	123	133	10
Average	88+	133	45−

We felt that possibly under the experimental conditions, anesthesia etc., it was impossible for the pressures to recover. For this reason other animals were given doses of 30 per cent sodium chloride or saturated Na_2SO_4 the day previous to experiment. The following day their pressures were determined. As we did not know their pressure before the ingestion of salt, the results are not of great value. The pressures found the next day can only be compared with our average normal pressures for animals under similar experimental conditions. The data bearing on this subject are given in table 2.

In these four experiments the average of pressures found many hours after salt ingestion is 88 mm., i.e., 45 mm. lower than our average of 133 mm. in the normal animal.

Unabsorbable salts. Experiments in which the unabsorbable salt, sodium sulphate, was used were also carried out. Changes qualitatively like those following sodium chloride occurred. The extent of change, however, and the rate at which it occurred were somewhat smaller in the case of the sulphate. Figure 5 is plotted from a sulphate ingestion experiment, a fall of 202 mm. occurred at the rate of 2.2 mm. per minute. Compare with figure 3, an experiment in which a large dose of sodium chloride was given. The average extent of fall after large doses of sulphate was 122 mm. at the average rate of 2.4 mm. per minute. Smaller doses caused roughly proportionate falls of pressure. As in the case of chloride ingestion, the low pressures endure for a considerable period.

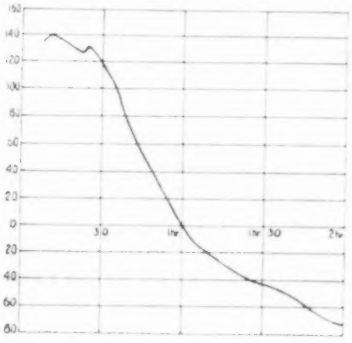

Fig. 5. Injection of 35 cc. saturated (120° F.) sodium sulphate solution into the duodenum. Cat weight 3.5 kgm. Chloretone anesthesia. A prompt fall of cerebro-spinal fluid pressure occurred—202 mm. at the rate of 2.2 mm. per minute. This rate is somewhat slower than in the case of similar doses of sodium chloride.

This ingestion of salt does not appear to make the animals refractory to a second dose at a later time. This was demonstrated by administering the salt on two successive days. Following the second administration the typical pressure changes occurred.

Glucose solutions. Concentrated solutions of glucose given into the gastro-intestinal tract are capable of producing qualitatively these same results, though quantitatively smaller in extent. Thus following the administration of 35 cc. of concentrated glucose solution into the duodenum the cerebro-spinal fluid pressure fell 140 mm. This is the largest fall we have obtained with dextrose solutions. The results are not as constant as with the salts.

Hypotonic solutions. The effects produced with hypotonic solutions are not nearly so striking. Several experiments were conducted in which water was injected into the duodenum. Thirty-five cubic centi-

meters caused rises of pressure averaging 45 mm. This rise is not very well sustained for within an hour practically normal pressure values obtained. Water seems somewhat more effective when it follows by several hours the ingestion of a hypertonic solution.

Alteration of brain bulk. A number of observations were made on the change of brain bulk following the administration of 30 per cent sodium chloride solution. The method employed was like that described by Weed and McKibben. The temporal muscles were dissected back and the dura with the underlying brain was exposed, through two trephine openings. The sodium chloride solution was injected into the duodenum. Within a short time following the administration of the solution the brain began to recede from the margins of the skull. Previously it had been bulging under the tense dura. The changes were very obvious, for within twenty to forty minutes the bulging had completely disappeared and the surface was concave. On incising the dura at this stage the sulci were found to be quite broad while the convolutions were contracted and narrow.

Proof of secretion and absorption pressure changes. The question naturally arises as to whether or not, in experiments like those recorded, the changes in the height of the manometer column may not be due simply to the dislocation of fluid from the manometer into the cerebrospinal fluid spaces coincident with the diminution in the size of the brain. That this factor plays some part in the pressure changes recorded, there can be no doubt for obviously as the subarachnoid spaces enlarge with a diminishing brain volume, fluid must run in to occupy them. On the other hand, there is very good evidence that the ingestion of these solutions causes alterations in the ratio between secretion and absorption of the fluid, producing pressure changes quite independent of brain volume changes. This fact was demonstrated by a number of experiments. With a certain dose of hypertonic solution the pressure changes of the cerebro-spinal fluid were recorded in a manometer of such bore that 100 mm. pressure change required a displacement of 0.26 cc. of fluid. The pressure changes following this same dose were then recorded in another animal using a larger bore manometer, in which 100 mm. of pressure change required a displacement of 1.06 cc. of fluid. Figure 6 shows the two curves obtained. If the whole process were merely one of change in brain volume, the second curve (large bore manometer, broken line) should illustrate a fall of pressure at a very much slower rate and very much less in extent, for the fluid displaced into the subarachnoid spaces would change the

height of the column in the small bore manometer a great deal more than in the large bore manometer. Such is not the case, for the new ratio established between secretion and absorption is capable of maintaining the pressure at a new level, independently of the volume of the cerebro-spinal fluid spaces or the size of the manometer used. A later report will be made of the remarkable changes which these solutions bring about in the absorption mechanism and in the currents of the fluid channels. They appear to be capable of reversing the flow in the perivascular spaces and the ventricular system.

The clinical significance of these problems is most apparent. They concern the states commonly referred to as "pressure symptoms." Up to the present the facts established by research have been more anatomical than physiological and it is significant that the clinical advances have been mostly applications of these facts and have little to do with physiology.

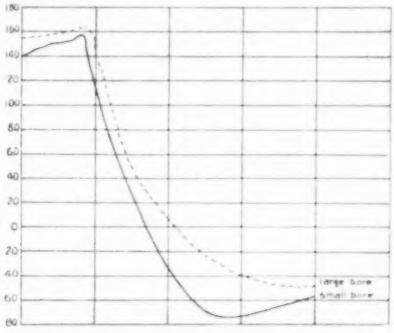

Fig. 6. Curves from two separate experiments. The broken line describes the cerebro-spinal fluid pressure as recorded in a large bore manometer, the continuous line describes the same pressure changes in another animal, but recorded with a small bore manometer. In each case the animal (cat) was given 10 cc. of 30 per cent sodium chloride solution intravenously.

SUMMARY AND CONCLUSIONS

1. Weed and McKibben demonstrated that it is possible to reduce the cerebro-spinal fluid pressure and diminish the bulk of the brain by injecting hypertonic solutions into the blood stream. Conversely, they showed that the pressure and the bulk of the brain could be increased by the injection of hypotonic solutions. Their work has been repeated and their general conclusions confirmed.

2. We have shown that the introduction of hypertonic salt solutions into the gastro-intestinal tract has a similar effect. This route of administration is more convenient, and by its use the disturbances of circulation and respiration common with intravenous infusions are avoided.

3. Twenty to thirty cubic centimeters of a 30 per cent sodium chloride solution introduced into the duodenum or rectum of an average

sized cat produced a maximal fall of cerebro-spinal fluid pressure. Following such doses, the average fall of pressure in a large series of experiments was 258 mm. of water. Larger doses added nothing to the extent of the fall. A dose of 5 cc. produced a fall of 104 mm. of water, and intermediate doses gave roughly proportionate results. Following this fall in pressure there is a gradual rise. Thus, seventeen to forty-eight hours after such injections, four animals showed pressures averaging 45 mm. less than the average normal.

4. Sodium chloride solutions in only slightly hypertonic concentration are also effective in causing a fall in cerebro-spinal fluid pressure, although to produce appreciable changes, large doses are required. Doses of 40 to 150 cc. of 2 per cent sodium chloride solution caused falls of pressure averaging 97 mm. of water, in cats.

5. Saturated solutions of sodium sulphate, which is not absorbed from the gastro-intestinal tract, produced qualitatively similar results, but less in extent and at a slower rate. With concentrated dextrose solutions the fall is still less and its rate still slower.

6. Water injected into the duodenum produces a small rise of pressure in the normal animal, but it disappears more rapidly than the fall incident to salt ingestion. If the animal has been given a concentrated saline solution the day before, the rise in pressure following the administration of water is more marked and of longer duration.

7. Such changes in cerebro-spinal fluid pressure were shown to be independent of changes in arterial or venous blood pressure.

8. These changes of fluid pressure are accompanied by a decrease in the size of the brain.

9. The manometer readings (pressure values) obtained after salt ingestion are not due solely to changes in brain volume and capacity of the cerebro-spinal fluid spaces, but primarily represent new ratios between secretion and absorption of cerebro-spinal fluid.

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ANTAGONISM OF DEPRESSOR ACTION OF SMALL DOSES OF ADRENALIN BY TISSUE EXTRACTS

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In a previous communication the writer (1) has shown that extracts made from heart, spleen, pancreas, testes, anterior and posterior lobe of the pituitary body, and the thymus, thyroid and parathyroid glands, in addition to stimulating the isolated uterus of the rat, guinea pig, virgin dog and cat, antagonized the inhibitory action of adrenalin on this tissue. It was also shown that the same extracts depress the cardiac vagus of the terrapin. It was demonstrated also that the pressor effect following the intravenous injection of a definite dose of adrenalin was augmented and longer maintained when splenic extract was previously injected. It was suggested that this latter result might be due to the depression or paralysis of some part of the vasodilator mechanism by the splenic extract.

Further investigation has confirmed the writer in this opinion.

Methods. The extracts used were made by boiling with distilled water in amount sufficient to give a practically isotonic solution, desiccated glandular products supplied by Armour & Company, Chicago; or else by extracting fresh tissue with distilled water on the water-bath, filtering and concentrating the filtrate to an aliquot volume of the original tissue.

The animals used for testing out the extracts were the dog and rabbit. The experimental animal was placed under ether anesthesia and a cannula was inserted into the left carotid artery. This was connected with the mercury manometer. Injections were made into the left external jugular vein by means of a "Record" syringe. If the fluid injected were small in amount the vein was at once flushed with Ringer-Locke's solution to insure that all the fluid should enter the general circulation.

Results. It was found that extracts of such widely divergent tissues as those of the spleen, skeletal muscle and parathyroid gland caused the pressor response of a definite dose of adrenalin administered by the intravenous route to be augmented and longer maintained. Thus it was found that 3 cc. of adrenalin, 1:50,000, injected into a dog weighing 14 kilos, produced a rise of 32 mm. in blood pressure with a return to normal in 40 seconds, whereas the same amount of adrenalin injected 5 minutes after the administration of 25 cc. of extract of ox spleen produced a rise of 52 mm. in blood pressure with a return to normal in 4 minutes. The injection of 0.5 cc. of 1:50,000 adrenalin into a rabbit weighing 2 kilos produced a rise of 54 mm. in blood pressure with a return to normal in 80 seconds while the same amount given after the injection of 5 cc. of extract of ox muscle produced a rise of 62 mm. in blood pressure with a return to normal in 3½ minutes.

In another instance a dog weighing 22 kilos with both vagi cut responded to 4 cc. of adrenalin 1:50,000 by a rise of 80 mm. in blood pressure with a return to normal in 1 minute 50 seconds; 20 cc. of extract of ox spleen were then injected and when the depressor action of this had passed off 4 cc. of adrenalin 1:50,000 were again injected. This produced a rise of 110 mm. in the blood pressure with a return to normal in 3 minutes.

An animal in which the depressor effect of small doses of adrenalin is readily elicited is most suitable to demonstrate the antagonism of this latter action by tissue extract. Figure 1 illustrates the antagonism of the vasodilator action of small doses of adrenalin by extracts of various tissues. The experimental animal in this instance was a male dog 25 kilos in weight. Shortly after ether anesthesia had been induced, the injection of 2 cc. of 1:50,000 adrenalin produced a fall of 22 mm. of mercury in the blood pressure (fig. 1, I). Both vagi were cut and the injection of 3 cc. of 1:50,000 adrenalin produced a fall of 32 mm. in the blood pressure. One cubic centimeter of 1:1,000 adrenalin was next injected and the blood pressure rose 86 mm. The injection of 3 cc. of 1:50,000 adrenalin then produced a slight rise in the blood pressure which was followed at once by a slight fall in the same with an immediate return to normal. This latter effect stands in sharp contrast with the effect of 3 cc. of 1:50,000 adrenalin prior to the injection of the stronger extract (fig. 1, A). After 10 minutes had elapsed the injection of 3 cc. of 1:50,000 adrenalin produced an effect similar to that following the first injection of 3 cc. Figure 1, B, shows the conversion of a fall of 16 mm. in blood pressure following the intravenous injection of 3 cc.

of 1:50,000 adrenalin into a rise of 20 mm. after the injection of 10 cc. of extract of thyroid gland.

Figure 1 (*C*, *D*, *E*, *F*, *G* and *H*) illustrates the same type of phenomenon induced by extracts of pancreas, thymus gland, corpora lutea, anterior lobe of pituitary, testes and parathyroid glands respectively.

This antagonism of the depressor action of small doses of adrenalin by extracts of various tissues was not of long duration. The primary effect of adrenalin in small doses given intravenously returned in from 10 to 30 minutes after the injection of the tissue extract (fig. 1, D and G).

Discussion. The antagonism of the depressor action of small doses of adrenalin and the augmentation and prolongation of the pressor response to the larger doses would appear to be phenomena of the same order. As Hartman (2) and others have shown, there is a definite vasodilator mechanism which is activated by adrenalin. Different opinions have been expressed as to the exact causation of adrenalin dilatation. Dale (3) was of opinion that small amounts of adrenalin stimulate the vasodilator endings. Cannon and Lyman (4) attributed the two effects, vasodilatation and vasoconstriction, to opposite actions according to the state of the muscle,—relaxation when tonically shortened, contraction when relaxed. Gruber (5) suggested that the central nervous system was involved in the dilatation from adrenalin. Hartman, Kilborn and Fraser (6) obtained vasodilatation in the hind limb and intestine by the central action of adrenalin. They concluded that adrenalin stimulates vasodilator cells situated in the sympathetic and posterior spinal root ganglia.

It would appear that the tissue extracts studied depress in some manner some part of the vasodilator mechanism. The effect of a small dose of adrenalin which normally produces a fall in blood pressure is thus neutralized in whole or in part by the tissue extract as far as the dilator action is concerned, and constriction only or else a lesser degree of dilatation takes place. When a larger dose of adrenalin is given, one such as causes a rise in blood pressure, the administration of tissue extract depresses the vasodilator apparatus and the vasoconstrictor effect is thus augmented and prolonged. Whether the point of action of this peculiar constituent of tissue extract is the nerve ending, nerve fiber or nerve cell of the vasodilator apparatus is undetermined. The writer inclines to the view that the action is peripheral since the antagonism of the inhibitory action of adrenalin on the uterus and the depression of the cardiac vagus of the terrapin produced by similar extracts was shown to be due to peripheral action (1). It is however possible that the vasoconstrictor

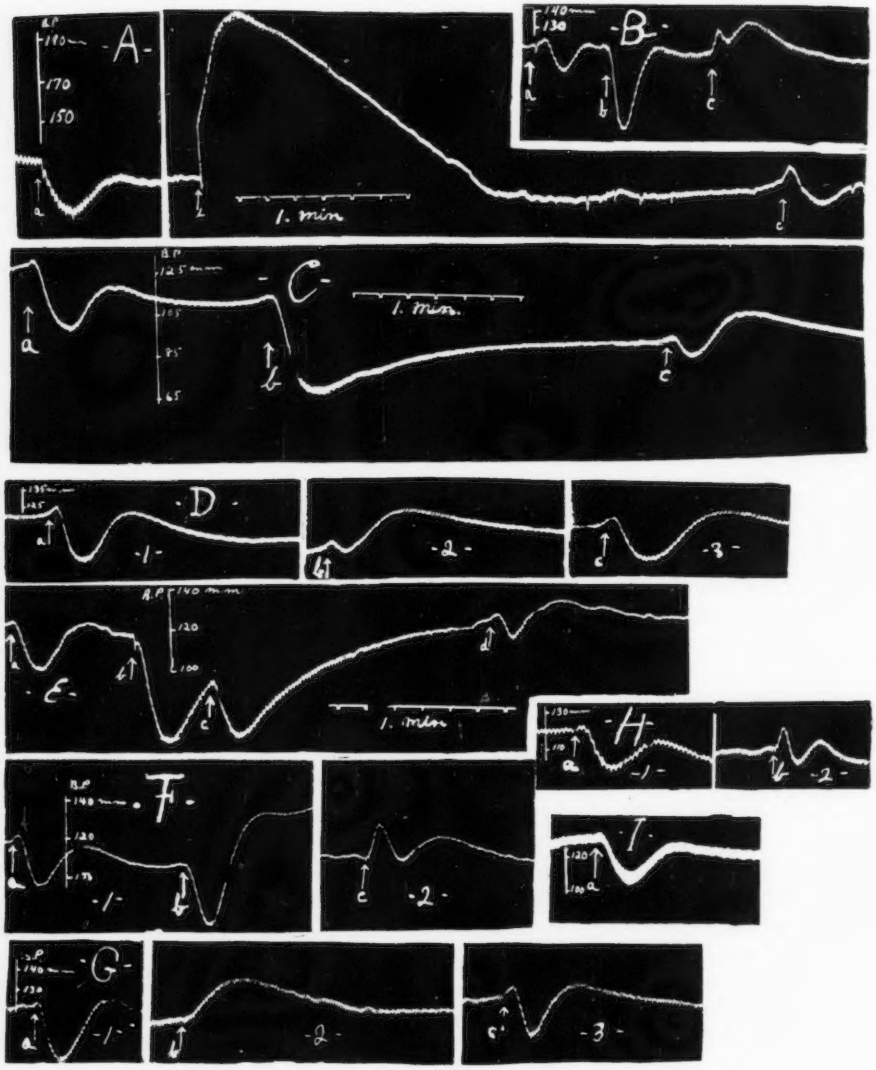

Fig. 1. Dog 0; 25 kilos. Ether anesthesia

A: *a*, 3 cc. of adrenalin 1:50,000 injected into left external jugular vein.
b, 1 cc. of adrenalin 1:1000 intravenous.
c, 3 cc. of adrenalin 1:50,000 intravenous.

mechanism may be rendered more sensitive to adrenalin by tissue extract.

The short duration of the antagonistic action of tissue extract, given by intravenous injection, upon adrenalin dilatation indicates that the active principle is either neutralized or destroyed fairly rapidly when present in the general circulation.

SUMMARY

1. The fall in blood pressure produced by a small dose of adrenalin is antagonized by various tissue extracts.

2. The rise in blood pressure produced by a definite dose of adrenalin is augmented and prolonged by administration of tissue extract.

3. It is held that both these types of effects are of the same order.

B: *a*, 3 cc. of adrenalin 1:50,000 intravenous.
b, 10 cc. extract of thyroid gland intravenous.
c, 3 cc. of adrenalin 1:50,000 intravenous.

C: *a*, 3 cc. of adrenalin 1:50,000 intravenous.
b, 20 cc. extract of pancreas intravenous.
c, 3 cc. adrenalin 1:50,000 intravenous.

D: *a*, *b* and *c*, 3 cc. of adrenalin 1:50,000 intravenous.
Between *1* and *2*, 20 cc. of extract of thymus gland injected into left external jugular vein.
3, five minutes after *2*.

E: *a*, 3 cc. adrenalin 1:50,000 intravenous.
b, 20 cc. of extract of corpora lutea intravenous.
c, 10 cc. of extract of corpora lutea intravenous.
d, 3 cc. of adrenalin 1:50,000 intravenous.

F: *a*, 3 cc. of adrenalin 1:50,000 intravenous.
b, 10 cc. of extract of anterior lobe pituitary intravenous.
c, 3 cc. of adrenalin 1:50,000 intravenous.
2, taken eight minutes after *1*.

G: *a*, *b* and *c*, 3 cc. of adrenalin 1:50,000 intravenous.
Between *1* and *2*, 40 cc. of extract of testes injected into left external jugular vein.
3, taken ten minutes after *2*.

H: *a* and *b*, 3 cc. of adrenalin 1 : 50,000 intravenous.
Between *1* and *2*, 10 cc. of extract of parathyroid glands injected into left external jugular vein.

I: *a*, 2 cc. of adrenalin 1:50,000 intravenous. Vagi intact.

Note: In all other instances vagi were cut.

4. The antagonism of the depressor action of adrenalin by tissue extract is of short duration.

5. Some part of the vasodilator mechanism is depressed by tissue extract.

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OBSERVATIONS ON A SEX DIFFERENCE IN THE PRESENCE OF NATURAL HEMOLYSIN IN THE RAT

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It has been often found that without previous treatment the serum of one animal can hemolyse the red blood corpuscles from an animal of another species. Such hemolysin is called "natural hemolysin." The instances of the presence or absence of natural hemolysins against different species of animals have been noted among the various animals which are commonly used in the laboratories. However, so far as I am aware, no systematic observations have been made with the corpuscles or serum of the rat in regard to the hemolytic activities of these fractions against those from other animals, and for this reason we have carried out the following investigation.

The technique used is as follows:—The rat was lightly etherized and the blood was collected from the carotid artery. The serum was diluted to ten times its volume and in each case 0.2 cc. was mixed with 0.5 cc. of 10 per cent washed blood corpuscles of the pig. To this mixture 1.3 cc. of normal salt solution was added, thus making up the total amount to 2 cc. This mixture was placed for half an hour in an incubator at 37°C. Sometimes a much greater dilution of serum was also tried, but unless otherwise stated the results refer to the ten-times dilution.

It was found in the first instance that the rat serum did not hemolyse the red blood cells of the pig; in other words the rat serum did not possess the anti-pig natural hemolysin. This result was obtained from the mixed sera of a dozen adult rats of both sexes combined.

However during further experimentation it was accidentally noted in one case that the serum of the rat used when mixed with the pig corpuscles hemolysed the latter readily. This particular rat happened to be a female immediately after parturition. Since we had previously found, as we thought, that the rat serum lacks the anti-pig hemolysin, but yet in this particular instance a highly active anti-pig hemolysin was found, we decided at once to repeat the observations. Table 1 gives the results of the further observations.

We find from table 1 that during the first five to six days after parturition the anti-pig hemolysin is present, but is less frequently present after this period. This interesting result induced us to study further. As a first step we tested for the presence or absence of anti-pig natural

TABLE 1

After parturition. Anti-pig natural hemolysins in female rat

DAYS AFTER PARTURITION	BODY WEIGHT	BODY LENGTH	AGE	HEMOLYSIN	REMARKS
	grams	*mm.*	*days*		
1				++	Normal
2 (2)				++++	Normal
2			150	+	Normal
3	171		90	+++	Normal
3			300	+	Normal
5	180		90	++++	Normal
5 (3)				−	Normal
6	168			++++	Slightly infected
9 (2)				−	Normal
10	115			−	Normal
11	171	184		++	Normal
16	131	179		−	Normal
25	165	183		−	Normal

TABLE 2

Natural anti-pig hemolysin in the rat serum under various conditions

Total number of rats examined = 211

PRESENCE OF ANTI-PIG NATURAL HEMOLYSIS IN RAT SERUM	FEMALES		MALES	
	Absent	Present	Present	Absent
	per cent	*per cent*	*per cent*	*per cent*
Pregnancy	35.8	64.2		
1 to 7 days after parturition	27.3	72.7		
7 to 25 days after parturition	83.3	16.7		
Normal adults	57.7	42.3	17.6	82.4
Normal young—under 50 days	87.5	12.5	14.3	85.7
Infected lungs	59.3	40.7	50.0	50.0

hemolysin of rats under various conditions, and obtained the results which are shown in table 2.

1. The number of cases in which the serum possesses the anti-pig hemolysin is greater among the females in pregnancy, during the first week after parturition, and in males in infection of the lungs.

2. The hemolysin is usually absent in rats of both sexes whose ages are less than fifty days.

3. Among the adult females the hemolysin is found in almost half the number of rats examined. Whether or not this high frequency was due to the use of rats at an early period of pregnancy cannot be determined. These females however had been with the males.

TABLE 3

Anti-pig natural hemolysin. Observations on the same animal at two or more ages

SEX	AGE AT THE TIME OF EXAMINATION				REMARKS
	30 days	70 days			
3 ♂	−	−			
♂	−	+			
♀	−	+++			
♀	−	++*			* Emaciated
♀	−	(+)			
♀	−	++			
	75 days	96 days			
♂	−	−			
♀	−	+*			* Pregnant
3 ♀	−	−			
	106 days	119 days	132 days	166 days	
♂	−	−	+ (sick)	+*	* Sick, killed
♀	++	++++			Sick, killed
♀	±	±			Died after operation
♀	−	−	−		
♀	−	+			Found dead
♀	+++	Not exam.	+++	+++	
	216 days	232 days			
♂	+	++			Sick, killed
♂	−	+			
♂	++++	+++			
2 ♀	−	−			
♀	−	+++			Sick, killed

4. Among normal males we find a comparatively small number of cases in which the hemolysin is present.

5. Among males with infected lungs, 50 per cent of the cases possessed the hemolysin.

We may conclude from these data that anti-pig natural hemolysin is far more frequently present in the female than in the male.

In order to verify the statistical findings, and also further to investigate whether or not the natural hemolysin appears suddenly associated with both pregnancy, as well as with lung infection, and also with the idea of determining whether or not the degree of titration increases with the advance of pregnancy or of infection, the following experiment was undertaken.

The rats at various ages were examined individually for the presence or absence of the anti-pig natural hemolysin. The same rats were again examined after a lapse of several weeks. In each instance the blood was obtained by puncture of the heart. As table 3 shows, this puncture was made in two instances at four different ages.

It was intended to carry such an examination throughout the entire span of life. However disturbances in the rat colony from ecto-parasites unfortunately prevented the completion of this work. Nevertheless the results obtained, though imperfect, show several interesting points, and these are presented in table 3.

From this table 3 we see that in many instances the hemolysin was not present at the earlier age but appeared later. In every instance where the hemolysin was found at the earlier age it was found later also.

DISCUSSION

Recently Kolmer and Casselman (1) examined a large number of human sera for natural hemolysins against the erythrocytes of the sheep, dog, ox, goat, hog, rat, chicken, horse, rabbit and guinea pig. They found a considerable variation in the activity of the human sera to these various blood cells. Kolmer and Casselman did not however mention any variation associated with either sex, disease or pregnancy.

While the present investigation was nearing completion, I received from Doctor Obata a paper (in Japanese) on comparative immunological studies on the fetal and adult sera in man. Doctor Obata found in man practically all that I have found in the rat; that is the anti-sheep natural hemolysin occurs more frequently in women than in men, and furthermore the hemolysin is not present in the fetal blood. Doctor Obata found however that the anti-sheep natural hemolysin is equally distributed among non-pregnant women and pregnant women, while my own observation on the rat shows a much greater frequency in both pregnancy and the first week after parturition than in the non-pregnant adult female.

From the foregoing observations I conclude that

1. Anti-pig natural hemolysin is not usually present in the serum of younger albino rats under thirty to fifty days of age.

2. In older rats this hemolysin may be present in the serum of both sexes but more frequently in that of the female than in that of the male rat.

3. During pregnancy, as well as during the first week after parturition, the anti-pig hemolysin is not only more active but the proportion of cases is far greater than among normal non-breeding females.

4. The number of cases in which the anti-pig hemolysin is present tends in males to increase with the appearance of lung infection.

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STUDIES ON ABSORPTION FROM SEROUS CAVITIES

III. The Effect of Dextrose upon the Peritoneal Mesothelium

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It has long been known that absorption from the peritoneal cavity takes place rapidly and effectively. The absorption of solutions and particulate matter has been studied by many investigators, but very little effort has been made to use the peritoneal cavity as a route by which therapeutic agents might be administered. The basis of practical intraperitoneal therapeusis must be founded upon three general factors: *a*, the physiological and toxicological effects on the organism of substances administered by this route; *b*, the variations upon tissue resistance caused by the administration of any substance; and *c*, the direct effect upon the mesothelium. The determination of the effect of any substance upon the organism must be carefully controlled for each method of administration, as it is well known that there is a great variation in the effect of the same substance given by different routes. Such methods need not be discussed here, since they are in constant and practical use. In the peritoneal cavity the effect upon the resistance of tissues which are directly exposed to the first shock of the administration should be considered with very great care.

Blackfan and Maxcy (1) have reported the use of the peritoneal route for the administration of physiological saline to patients requiring additional fluids, with much success and no apparent ill effects. In their report they also give animal experiments in which they found rapid absorption of the saline; the peritoneum remaining smooth and glistening, no adhesions forming, and no abrasions of the viscera taking place. They have also shown that the introduction of salt solution is attended with no risk of infection if reasonable surgical precautions are taken, but they have not controlled their experiments by a study of the comparative resistance of normal animals, with animals which have been subjected to the intraperitoneal injection of repeated doses of

physiological saline. This is obviously easy to determine with regard to any given substance, and any given exposure to that substance.

There has heretofore been no method for an early and certain diagnosis of injuries, or at least changes in the mesothelium. The formation of adhesions and an aseptic inflammation could of course be considered as definite criteria, but these are at best crude methods, and only indicate comparatively late stages of change in the mesothelium. It is now possible to estimate the effect of any given substance since it has been determined that definite morphological changes in the mesothelium can be produced by the repeated administration of certain solutions and suspensions. These changes have been produced by the introduction of particulate matter, laked blood and soluble starch. A preliminary note on these experiments has been published (Cunningham (2)); the detailed report has not been published but is in preparation. These findings seem to offer a possible criterion for the comparison of the effect which any substance will have upon the peritoneal mesothelium.

Dextrose was selected for study in this connection because, if it could be administered via the peritoneum without injurious effects, selection of that route would eliminate many difficulties incident to administration in other ways. The purpose of this contribution is not directed to the settlement of the action and fate of dextrose in the circulation, or to the reaction of the organism to this substance, but simply to the effect which it might have upon the mesothelium of the peritoneal cavity.

Technique and material. Rats were used for these experiments. The animals were anesthetised with ether, the abdomen shaved and washed with alcohol, and then a drop of tincture of iodine applied. The dextrose solution was introduced by means of a 10 cc. record syringe, a fresh needle being used for each animal. The syringe and needles were sterilized by boiling. The dextrose, Merck's "pure," was prepared by dissolving in freshly distilled water and sterilizing in the Arnold for one hour on each of two successive days. The solutions were never sterilized under pressure, because this usually caused the breaking down of the dextrose molecule. Cultures of these solutions made at the time of administration were uniformly sterile.

Two series of animals are to be reported at this time. The first consisted of twelve rats, each of which received 10 cc. of a 10 per cent solution of dextrose; seven of these were killed at two, four, six, eight, ten, twelve and fourteen hours respectively after the injection. The other five were killed at intervals between ten and fourteen hours; this

was done in order to determine more exactly the time at which the fluid had been entirely absorbed. At autopsy cultures were taken, and the fluid in the peritoneal cavity was removed with a pipette; by this means practically all the fluid present could be recovered. Quantitative determinations of the sugar content were not done, because the actual rate of absorption of the sugar was not required for the present purpose. This will be referred to later. Tissue was fixed in Bouin's fluid, and prepared for section in the usual manner.

The second series consisted of twelve rats which received 10 cc. of a 10 per cent solution of dextrose every twenty-four hours for fourteen days. Six of these were killed at two, four, six, eight, ten and twelve hours respectively after the last injection. Three were killed at short intervals after twelve hours, in order to determine the end point of absorption as in the first series. The other three were kept as controls, and will be referred to later.

Experimental results. Figure 1 represents the amount of fluid found in the peritoneal cavities of the animals in the first series, while figure 2 is a similar curve for the second series. From these curves it is evident that the absorption of dextrose from the peritoneal cavity is not impaired by repeated doses of this solution, but the differences between the two curves are not great enough to establish conclusively that the exposure increases the ability of the peritoneum to absorb.

Autopsies on animals of series I revealed no changes in the peritoneal cavity, while examination of those in series II showed a slight brownish tint in the *taches laiteuses* of the omenta; otherwise they were entirely normal. No adhesions were found in any of the animals and the folds of the omenta were normal and free in every case. Usually three or four points of injection were visible as red areas about 1 mm. in diameter on the anterior abdominal wall; those from earlier punctures were almost entirely healed. Cultures taken at autopsy were sterile.

On histological examination of tissue from all the animals in the first series, the serous mesothelium appeared everywhere entirely normal and no changes were found in the *taches laiteuses*. On the other hand in the animals in the second series, which had received repeated doses of the dextrose solution, the mesothelium of the diaphragm, the spleen, the omentum, and in one case even the body wall, showed quite decided changes. The diaphragm and spleen always showed the most marked changes, which varied very little in the animals of the group, but the changes in the mesothelium of the omentum were much more variable, even in the same animal, while the mesothelial cells of the body wall

showed slight increase in size in only one animal. This distribution of changes corresponded very closely to that found after the use of laked blood, and was most interesting in that the diaphragm and spleen were always most markedly affected, the omentum somewhat, while the intestines, the liver, and in most cases the body wall, showed little or no change.

The mesothelial cells of the diaphragm and spleen showed everywhere a definite increase in size, the nuclei having become rounded and vesicular, and the cytoplasm increasing considerably in thickness. The borders of the cells were often withdrawn from the neighboring cells, so that a small area of the subjacent connective tissue seemed to be uncovered. Here and there, especially over the diaphragm, a few cells

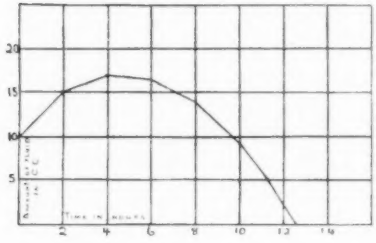

Fig. 1. Curve showing amount of fluid in peritoneal cavities of normal rats after introduction of 10 cc. of a 10 per cent solution of dextrose.

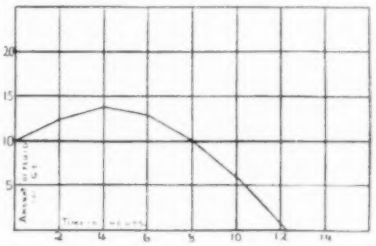

Fig. 2. Curve showing amount of fluid in peritoneal cavities of rats which had received fourteen injections of 10 cc. of a 10 per cent solution of dextrose.

had become almost separated from the underlying structures and were attached by only a small pedicle. Areas scattered over the diaphragm, which represent the space normally occupied by two or three cells, were bare; the cells evidently having desquamated. In the splenic mesothelium desquamation had not taken place to any great extent, although an occasional cell area was bare. Perhaps the most remarkable fact noticed was that, in the mesothelium of the diaphragm especially, but also in a less noticeable degree in the case of the spleen, many of the cells which had rounded up and increased in size were undergoing division. Mitotic figures could often be seen in several stages. In one section three figures were found in the area covered by the oil immersion lens. The cytoplasm of the mesothelial cells became much more basophilic as they rounded up and increased in size, but in no case could any granules in the cytoplasm be detected. Pycnotic nuclei were

never seen in any of these enlarged cells. One other interesting observation must be recorded; these large cells when carefully studied under very high magnification invariably presented a surface which was covered with fine projections simulating in appearance the cilia of normally ciliated cells. They differed from true cilia in that they varied considerably in length and width, and were somewhat irregular.

After examination of sections had demonstrated that dextrose produced such definite changes, other experiments were performed in order to determine how rapidly these changes occurred. Rats were given 10 cc. of dextrose solution and killed after one, two, three, four and five days respectively. After twenty-four hours no change was noticed. After forty-eight hours the only changes were a very slight thickening of the cell-bodies and a delicate irregularity of the peritoneal surface of the mesothelium, which suggested that the cells were beginning to form the cilia-like projections described above. After three or four days the cells had increased in size, and had become somewhat cuboidal in shape; while here and there some of them had begun to round up and the projections had increased. After the fifth injection the cells had rounded up and the free surfaces were covered with the characteristic cilia-like formations, but no evidence of any separation or desquamation was apparent.

Sections of the omenta of animals in the second series showed that the brown coloration mentioned earlier was due to a few fine granules of brown material in the cells of the *taches laiteuses*. These granules were very few in number and were scattered quite widely. There was no apparent change on careful comparison of sections of normal omentum with those of the rats in series II except for these few fine granules. Sections of anterior mediastinal lymph glands were examined to determine whether or not there was sufficient granular material in the sugar solution to have produced the changes noted in the mesothelium; but only an occasional granule could be found in any of the cells, which are usually loaded with them, when particulate matter has been absorbed from the peritoneal cavity.

In order to determine the effect of rest after exposure to dextrose the three rats, saved as controls from the series of animals which had received fourteen doses, were killed six, eight and ten days after the last dose was given. In all of these, sections demonstrated that the mesothelium was everywhere absolutely normal in appearance. From this it was evident that recovery had taken place completely in six days.

DISCUSSION

These experiments do not settle the question of the feasibility of using the peritoneal cavity as a route for the administration of dextrose; but they furnish certain evidence which is of value, both in regard to this practical application and to the general reactions of the peritoneal mesothelium. The experimental results which are favorable for the use of this method are: the general effect upon the animals, the nature of the curve of absorption, the rapid recovery of the mesothelium, and the absence of adhesions. But the changes in the mesothelial cells can not be considered as directly favorable, and may even prove a definite contraindication.

The animals suffered no ill effects as far as could be judged from their activity, general appearance and weight. While the rats were not weighed every day, their weights were taken before the first injection, and occasionally thereafter; none were found to have lost weight, while several actually gained a little. In studying the two curves it is evident that the length of time required for the complete absorption of the dextrose solution was about the same in both series of animals; but the increase in fluid, soon after the injection, was considerably greater in the first than in the second. From these observations it is evident that the continued exposure of the surfaces of the peritoneum did not produce sufficient injury or change to decrease their ability to absorb this particular solution.

In addition to this conclusion these curves suggest the discussion of certain general features of absorption, concerning which we have very little conclusive evidence. Immediately after the injection of the sugar solution the total amount of fluid in the peritoneal cavity increases, and this can only be at the expense of the water in the tissues and blood stream. At the same time we may conclude that the sugar is passing out of the peritoneal cavity into the blood stream and tissue spaces, although this has not been established in these experiments by quantitative examination of the fluid.

In short an equilibrium is being established by osmosis and diffusion between the two systems: the sugar solution which has been introduced into the peritoneal cavity, and the contents of blood vessels and tissue spaces. In considering the absorption of the solution after the equilibrium has been reached, which is indicated by the highest points of the curves, it seems evident that some other factor besides osmosis and diffusion must be involved. The most plausible explanation for this remarkable absorption, which takes place against the concentration gradient, seems to be that the sugar molecules are drawn over into the

blood stream and tissue spaces by means of some difference in electrical potential; and this increase in concentration of the dextrose in the blood forces the water out of the peritoneal cavity. The evidence obtained from these experiments is insufficient to establish any hypothesis regarding the nature of this exchange.

The nature of the change in the mesothelium has not yet been established, and upon this depends whether or not the reaction of these cells can be considered as a definite contraindication to the administration of dextrose via the peritoneal cavity. If the change is fundamentally an injury, if the desquamating cells are badly injured or dying, and if those remaining are unable to recover the denuded areas, then adhesions are likely to form and inflammatory processes to develop, if any organism should obtain access to the peritoneum. On the other hand if the change in these cells is the result of stimulation, if the cells are actively proliferating, if those cells which do separate are viable and continue life as free macrophages, and if the remaining cells are capable of renewing the denuded spaces so that only a few cell areas will be empty at any time, then adhesions should not develop and the protection against adventitious infection would seem likely to be as great as ever. Theoretical possibilities must include combinations of stimulation and injury, in which case any application would depend upon the ratio between the two.

These questions are most difficult to settle experimentally; the evidence obtained from these experiments tends to support the suggestion that the changes are due to stimulation. The mesothelium, after exposure to repeated doses of a 10 per cent solution of dextrose during a period of fourteen days, does not show denuded areas larger than the space normally occupied by two or three cells, there is no evidence of injury or death, but rather of active proliferation in these cells, no adhesions have occurred, and recovery, either by the return to normal of the cells which have rounded up or their replacement by other cells, is complete in six days.

The distribution of this change over diaphragm, spleen and omentum, while the remainder of the peritoneal surfaces is comparatively normal, would indicate that there is some special differentiation of the mesothelium in these regions. And it is quite possible that the explanation of the observed reaction should be sought in this differentiation rather than in some injurious effect produced on the cells by the dextrose.

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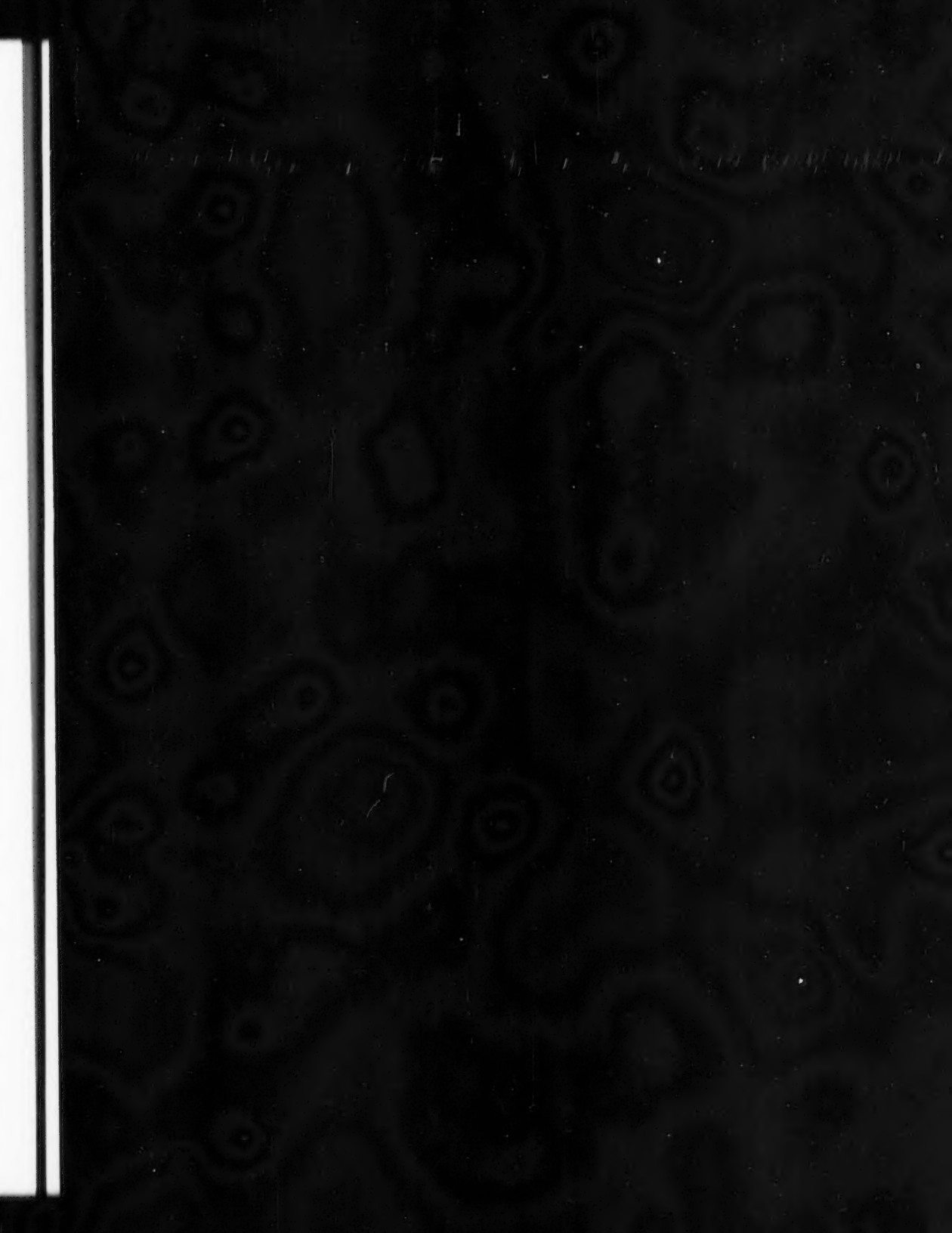

Adrenalin in Medicine

2—Treatment of the Paroxysm of Asthma

THE fact that Adrenalin promptly relieves the paroxysm of bronchial asthma has been demonstrated in thousands of cases. Explanation of its mode of action, however, must be couched in the language of probability and speculation, because the pathogenesis of the disease is the subject of an ever-increasing number of theories and much controversy.

Among the more reasonable and credible of these theories are: 1, Anaphylactic manifestations in the bronchial mucosa from bacterial protein sensitization; 2, The same condition produced by sensitization to food proteins (allergy), pollens of plants and animal emanations; 3, Reflex vagus irritation of the bronchial mucosa from peripheral afferent impulses originating along the course of distribution of this nerve.

It is not unlikely that every case of bronchial asthma can be explained by one of these theories, and that, indeed, in some of the cases more than one of these factors are underlying. Regardless of the theory or theories applicable to any given case, the immediate mechanical cause of the distressing paroxysm is a sudden spasmodic stenosis of the bronchioles. The action of Adrenalin is to relieve this stenosis. Whether the dilator muscles of the straitened tubules are stimulated or the circular constrictor muscles are temporarily paralyzed by Adrenalin to bring about this change in the calibre of the bronchioles cannot be definitely stated. It is interesting to note in connection with the protein sensitization theory that anaphylactic phenomena elsewhere in the body are often favorably influenced by Adrenalin—especially in respect to the skin manifestation, urticaria.

Adrenalin is the best emergency remedy for the treatment of the asthmatic paroxysm at the command of the physician. Two to ten minims of Adrenalin (1:1000) are given subcutaneously, or preferably intramuscularly. Frequently only five or ten seconds elapse after the injection when partial alleviation of the dyspnœa is noticed. In a few minutes relief is complete. Adrenalin acts quickly or not at all. In those few cases in which no favorable effect becomes apparent after the first injection this medication should not be pushed. Some practitioners have noted that the injection of Pituitrin in combination with Adrenalin (equal parts) enhances and prolongs the action of the latter.

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